

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: quma@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

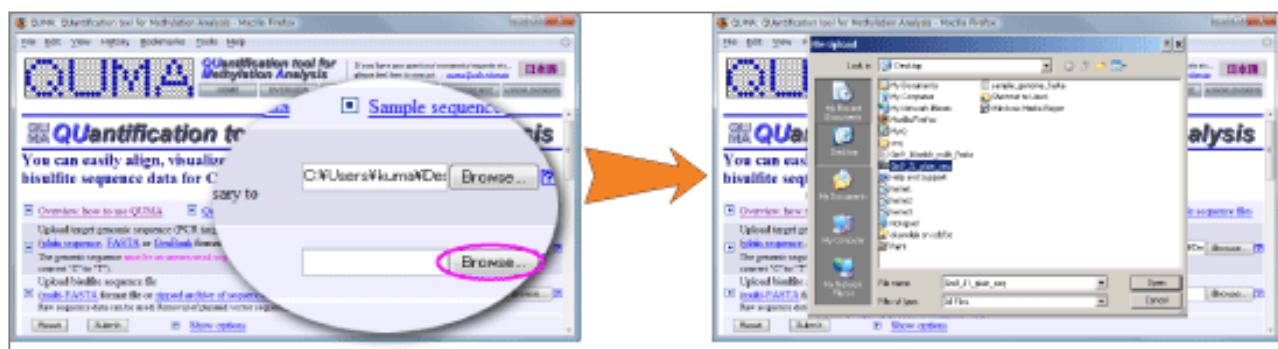
2.1. Select a genomic sequence file

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.)



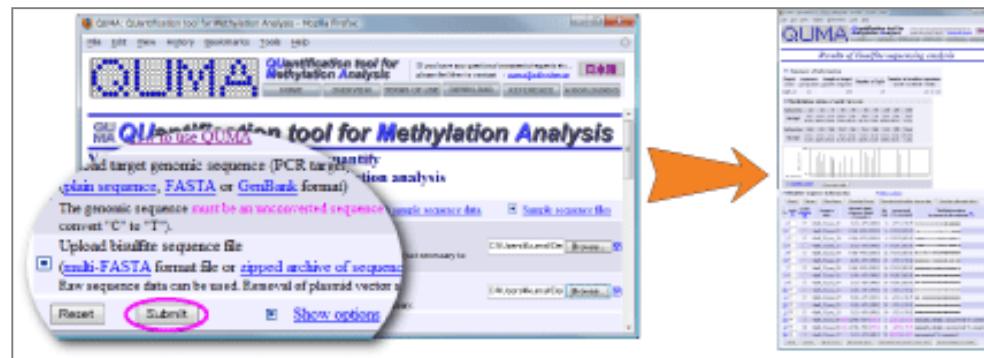
2.2. Select a bisulfite sequence file

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.

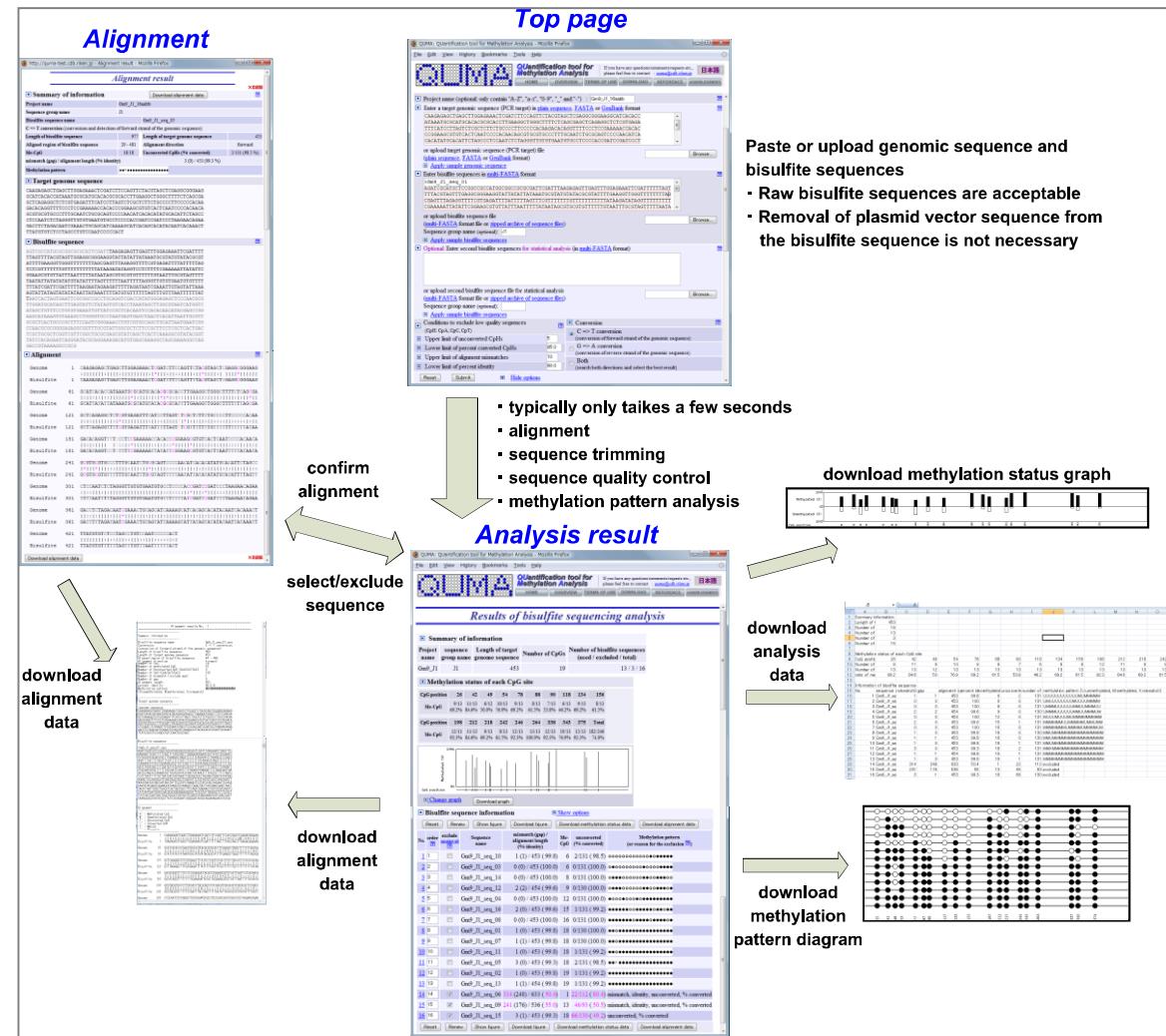
The screenshot displays the QUMA web application across three main sections:

- Top page:** Shows the "Quantification tool for Methylation Analysis" interface. It includes fields for "Project name" (J1_16sub), "Length of target genome sequence" (453), and "Number of bisulfite sequences (used / excluded / total)" (19 / 13 / 316). It also features sections for "CpG positions" and "CpG contexts".
- Methylation status analysis:** This section is titled "Results of bisulfite sequencing analysis". It shows a table of CpG positions and their context counts, followed by a bar chart of methylation status (M0, M1) for each position. Below the chart is a "Sequence viewer" showing the bisulfite sequence information for each position.
- Statistical analysis:** This section is titled "Results of statistical analysis". It displays a table of statistical results for Fisher's exact test and a bar chart of P-values. The table includes columns for "group 1" and "group 2" with their respective lengths and CpG counts.

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.



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](#).

The screenshot shows the QUMA web interface. At the top, there is a navigation bar with links for File, Edit, View, History, Bookmarks, Tools, and Help. Below the navigation bar is a logo consisting of a grid of blue dots forming the letters "QUMA". To the right of the logo, the text "QUantification tool for Methylation Analysis" is displayed in blue. Further to the right, there is a contact email address "guma@cdb.riken.jp" and a Japanese language link "日本語". Below the header, a large banner features the text "QUantification tool for Methylation Analysis" in a large, bold, blue font. Underneath the banner, a subtext reads: "You can easily align, visualize and quantify bisulfite sequence data for CpG methylation analysis". Below this, there are four checkboxes with corresponding links: "Overview: how to use QUMA", "Quick start", "Execute with sample sequence data", and "Sample sequence files". There is also a "Browse..." button and a question mark icon. A note states: "Upload target genomic sequence (PCR target) file (plain sequence, FASTA or GenBank format)". Another note says: "The genomic sequence must be an unconverted sequence between PCR primer pair (not necessary to convert "C" to "T").". Below this, another section asks for "Upload bisulfite sequence file" and provides a link: "(multi-)FASTA format file or zipped archive of sequence files)". A note states: "Raw sequence data can be used. Removal of plasmid vector sequence is not necessary.". At the bottom of the form, there are "Reset" and "Submit" buttons, and a "Show options" link.

5.3. Top page simple

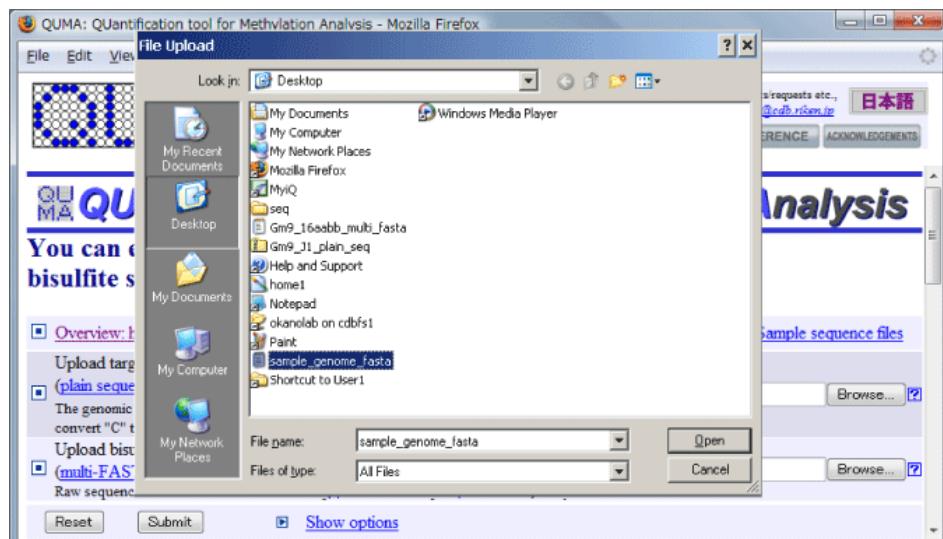
5.3.1. Genomic sequence file 1

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](#)".



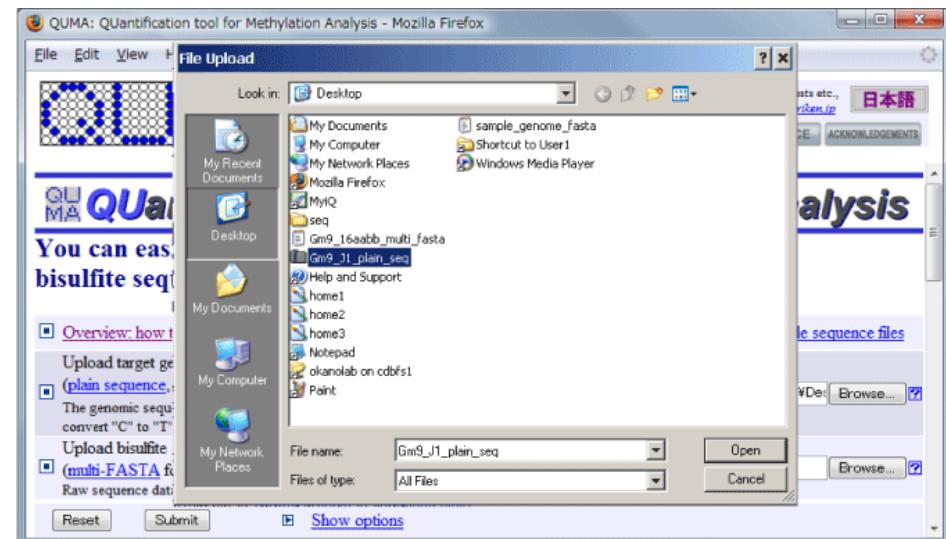
5.3.3. Bisulfite sequences file 1

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 shows the QUMA web interface with the title "QUantification tool for Methylation Analysis". The main menu includes File, Edit, View, History, Bookmarks, Tools, and Help. A "HOME" button is visible. On the right, there are links for "OVERVIEW", "TERMS OF USE", "DOWNLOAD", "REFERENCE", and "ACKNOWLEDGEMENTS". A Japanese language icon is present. The main content area has a large "QUMA" logo. Below it, several optional fields are shown:

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

Below these are two input fields:

- 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)

Below these are three more input fields:

- 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)
or upload second bisulfite sequence file for statistical analysis ([multi-FASTA](#) format file or [zipped archive of sequence files](#))
Sequence group name (optional):
 Apply sample bisulfite sequences
- Conditions to exclude low quality sequences
 - (CpI: CpA, CpC, CpT)
 Upper limit of unconverted CpHs:
 - Lower limit of percent converted CpHs:
 - Upper limit of alignment mismatches:
 - Lower limit of percent identity:

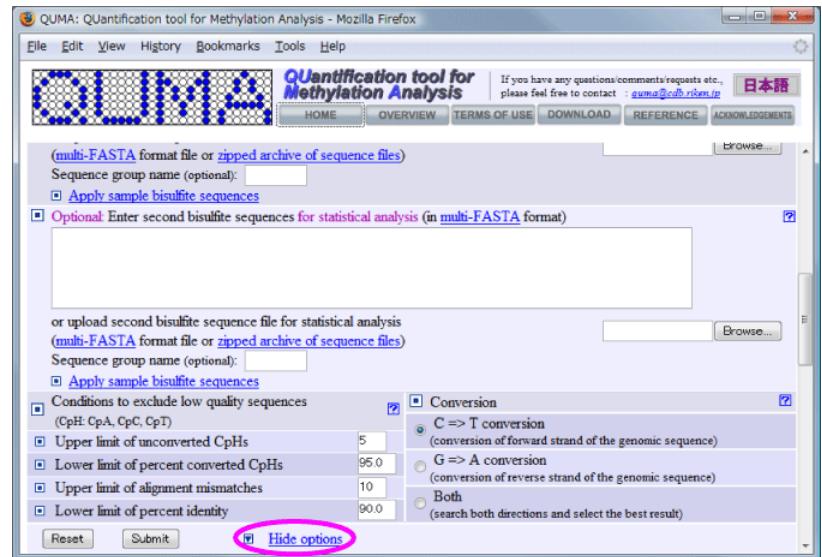
On the right side, there are conversion options:

- Conversion
 - C => T conversion (conversion of forward strand of the genomic sequence)
 - G => A conversion (conversion of reverse strand of the genomic sequence)
 - Both (search both directions and select the best result)

At the bottom are buttons for "Reset", "Submit", and "Hide options".

5.4.3. Hide options

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

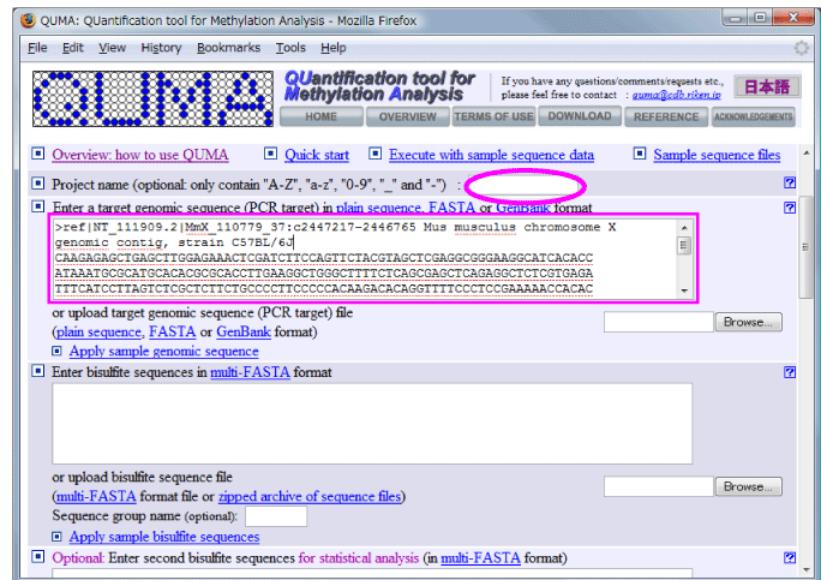


5.4.4. Genomic sequence

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](#)".



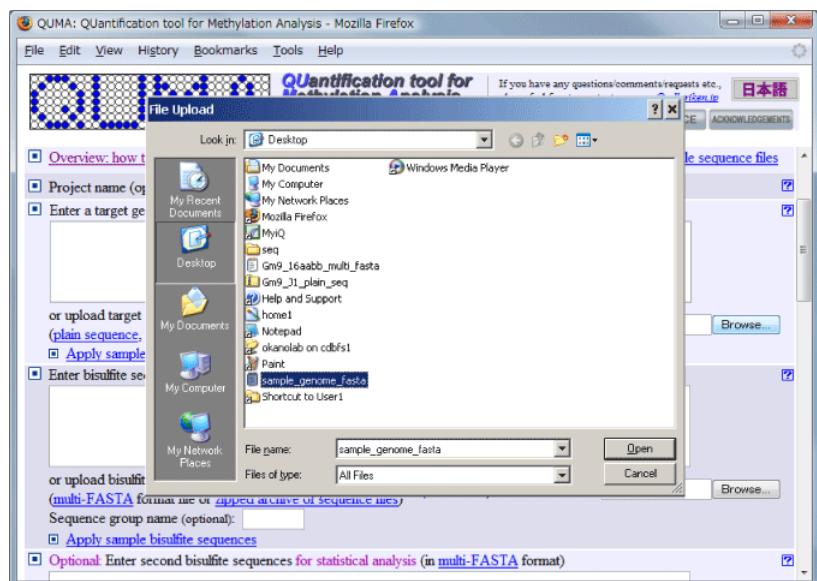
5.4.5. Genomic sequence file 1

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](#)".

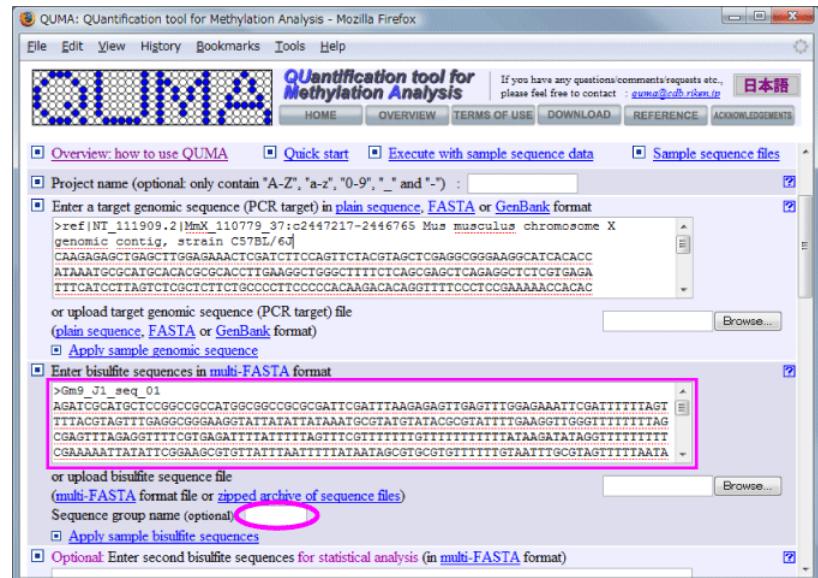


5.4.7. Bisulfite sequences

Input a group name of bisulfite sequences (optional).

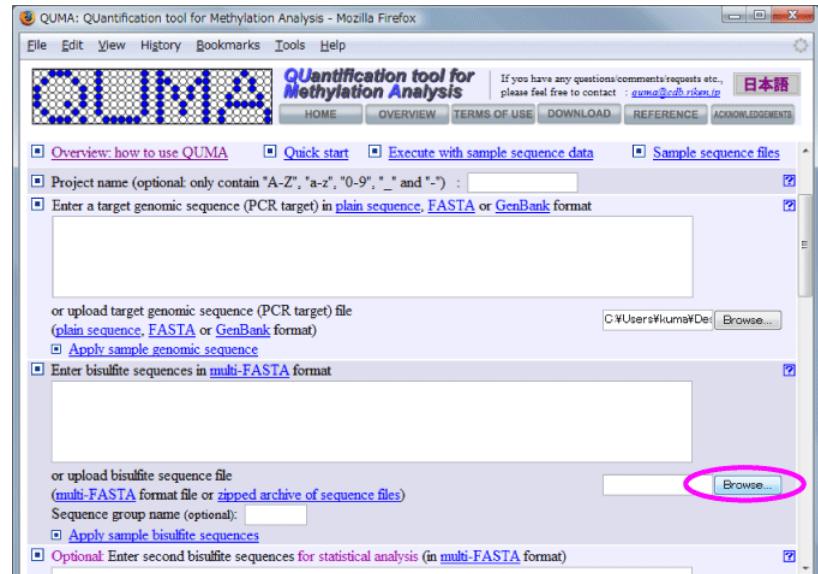
The bisulfite sequences can be input by two ways of 1) direct input and 2) upload.

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



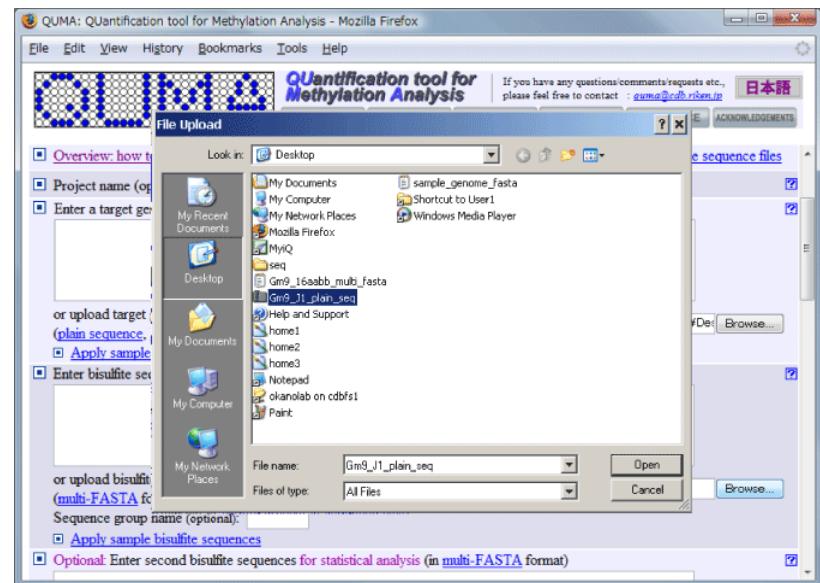
5.4.8. Bisulfite sequences file 1

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



5.4.9. Bisulfite sequences file 2

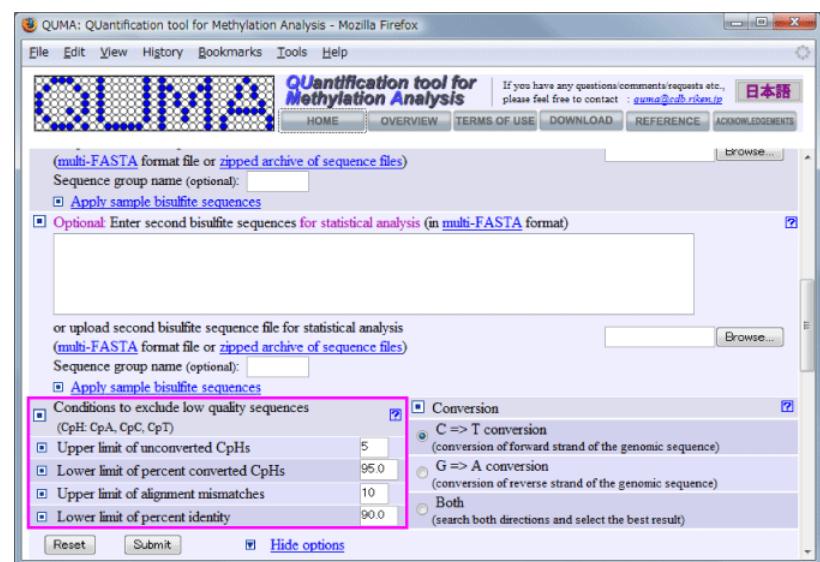
Select a file of bisulfite sequences. Acceptable file formats are [8.4. Multi-FASTA](#) or [8.5. Zipped archive of sequence files](#). See also "[7.2. Bisulfite sequences](#)", "[8.6. How to create zipped archive \(Macintosh\)](#)" and "[8.7. How to create zipped archive \(Windows\)](#)".



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



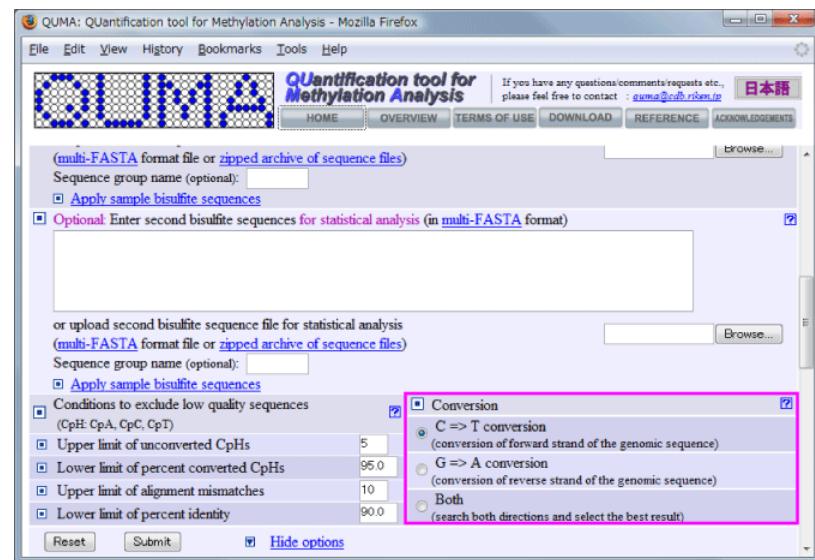
5.4.11. Strand of bisulfite conversion

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



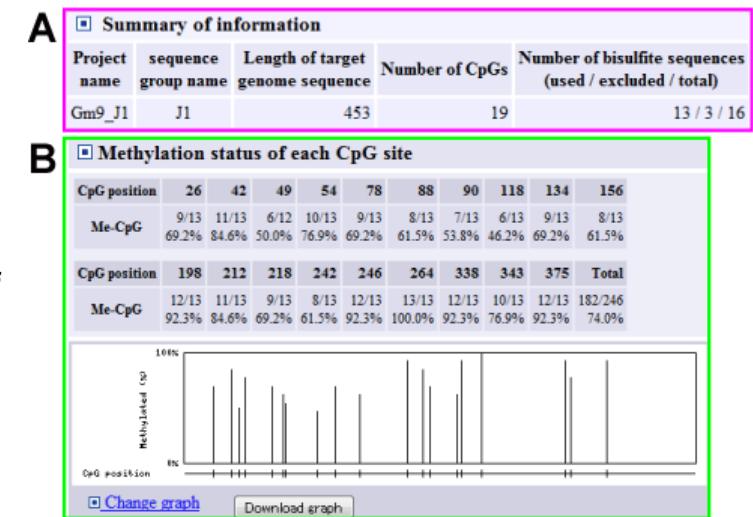
5.5.2. Overview of analysis result page 2

A) Summary of information

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

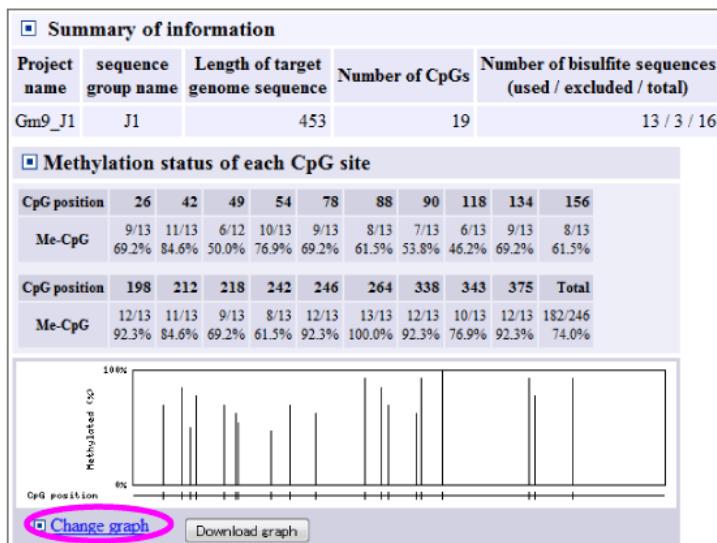
B) Methylation status of each CpG sites

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



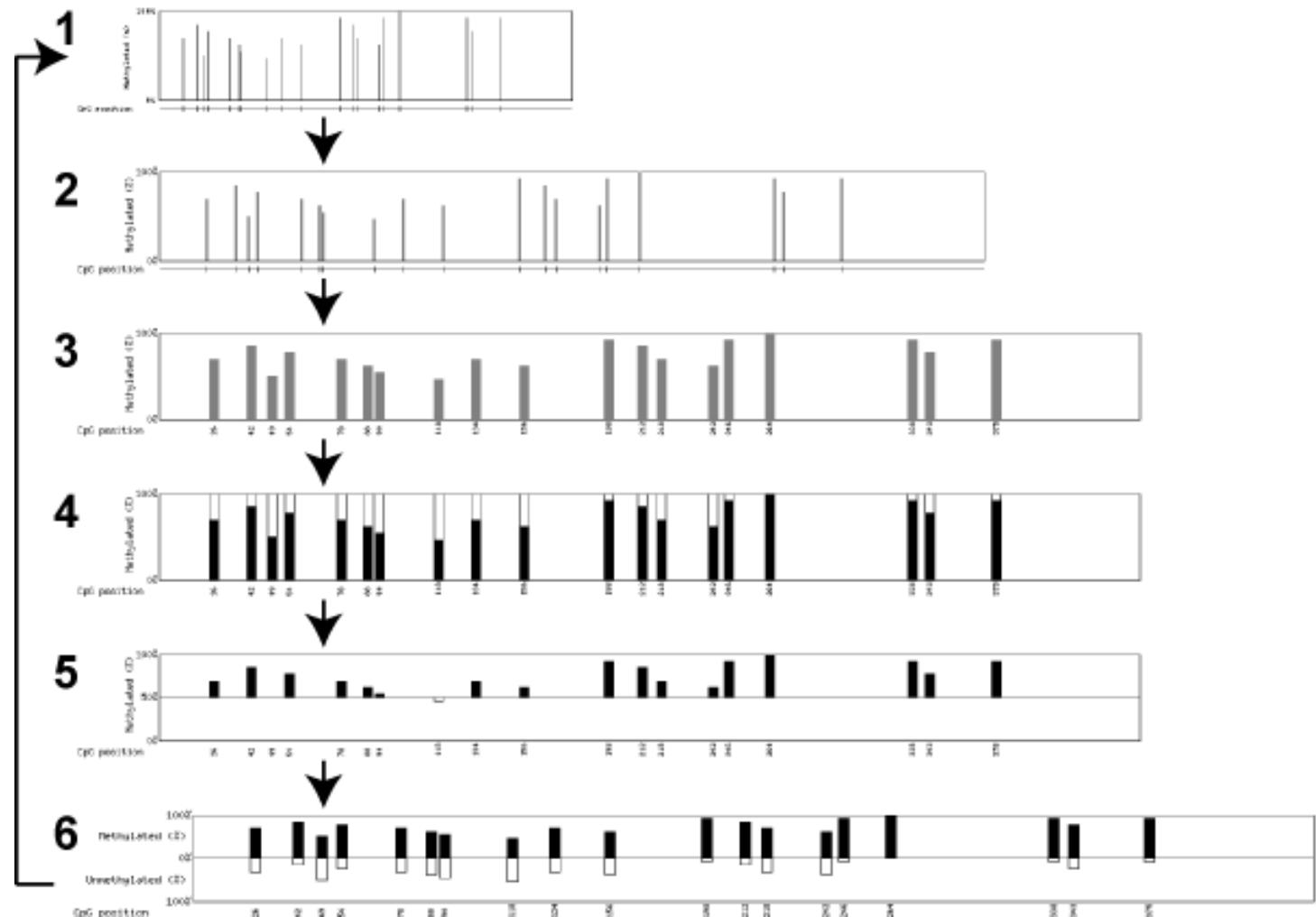
5.5.3. Change methylation status figure 1

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



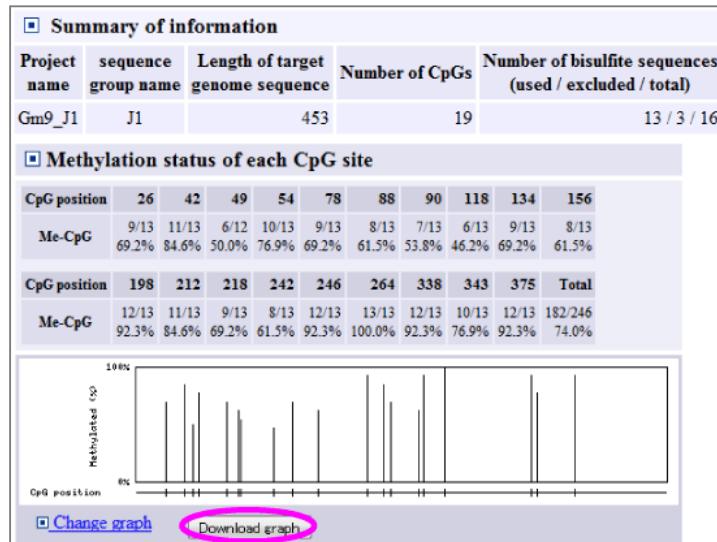
5.5.4. Change methylation status figure 2

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.



C Bisulfite sequence information Show options

No.	order	exclude <input type="checkbox"/> <small>unselect all</small> <input type="checkbox"/>	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion <input type="checkbox"/>)
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)	oooooooooooo*oooo*
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooo*oooo*
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooo*oooo*
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooo*oooo*
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	*****oooooooo*oooo*
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	*****oooooooo*oooo*
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	*****oooooooo*oooo*
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	*****oooooooo*oooo*
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	*****oooooooo*oooo*
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	***xoooooooo*oooo*
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	*****oooooooo*oooo*
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	*****oooooooo*oooo*
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

5.5.6. 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 CpHs (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).

- 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
No.	order	exclude <input type="checkbox"/> unselect all	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)	oooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	xxoooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooo
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

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
No.	order	exclude <input type="checkbox"/> unselect all	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)	oooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooo
11	11	<input checked="" type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	xxoooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooo
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

5.5.9. Include/exclude bisulfite sequence 2

The change is reflected.

Bisulfite sequence information						
No.	order	exclude sample call	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5) ○oooooooooooo●oooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0) ○oooooooooooo●oooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0) ○●oooooooooooo●oooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0) ○●oooooooooooo●oooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0) ○○○oooooooooooo●oooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2) ●oooooooooooo●oooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0) ●oooooooooooo●oooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0) ○oooooooooooo●oooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0) ○oooooooooooo●oooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2) ●oooooooooooo●oooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2) ●oooooooooooo●oooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2) ●oooooooooooo●oooooooo
13	13	<input checked="" type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5) used 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

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						
No.	order	exclude sample call	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)
1	6	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5) ○oooooooooooo●oooooooo
2	5	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0) ○oooooooooooo●oooooooo
3	4	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0) ○●oooooooooooo●oooooooo
4	3	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0) ○●oooooooooooo●oooooooo
5	2	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0) ○○○oooooooooooo●oooooooo
6	1	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2) ●oooooooooooo●oooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0) ○oooooooooooo●oooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0) ○oooooooooooo●oooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0) ○oooooooooooo●oooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2) ●oooooooooooo●oooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5) ○●oooooooooooo●oooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2) ●oooooooooooo●oooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2) ●oooooooooooo●oooooooo
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

5.5.11. Change the order of bisulfite sequences 2

The change is reflected.

Bisulfite sequence information						
No.	order	exclude under all	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (%) converted)
1	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	0/131 (99.2)
2	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)
3	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)
4	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)
5	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)
6	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)
7	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)
8	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)
9	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)
10	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)
11	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)
12	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)
13	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)
14	<input checked="" type="checkbox"/>	<input 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"/>	<input 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"/>	<input type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2) unconverted, % converted

5.5.12. Download alignments data

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

Bisulfite sequence information						
No.	order	exclude under all	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (%) converted)
1	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)
2	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)
3	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)
4	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)
5	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)
6	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)
7	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)
8	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)
9	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)
10	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)
11	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)
12	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)
13	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)
14	<input checked="" type="checkbox"/>	<input 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"/>	<input 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"/>	<input type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2) unconverted, % converted

5.5.13. Alignments data

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

5.5.14. Download analysis data

Click "Download methylation status data" button to download analysis data.

Bisulfite sequence information							Show options
No.	order	exclude methylated sites	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)	oo-----oooooooooooo
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 (<u>50.4</u>)	1	22/112 (<u>60.4</u>)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (<u>55.0</u>)	13	46/93 (<u>50.5</u>)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (<u>49.2</u>)	unconverted, % converted

5.5.15. Analysis data

Downloaded analysis data file can be opened by Microsoft Excel, [OpenOffice/StartSuite](#) or other spreadsheet software (**CSV** file format).

See also “[10.1. How to open a CSV file](#)”.

5.5.16. Download methylation pattern figure

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

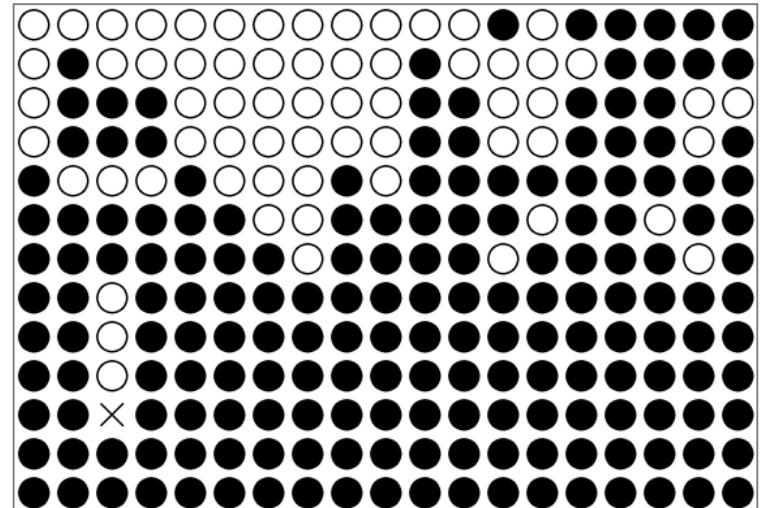
Bisulfite sequence information							<input type="checkbox"/> Show options
No.	order	<input checked="" type="checkbox"/> <input type="checkbox"/> all	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion <input type="checkbox"/>)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	ooooooooooooo•oooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	ooooooooooooo•oooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	ooooooooooooo•oooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	ooooooooooooo•oooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	ooooooooooooo•oooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	ooooooooooooo•oooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	ooooooooooooo•oooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooo•oooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooo•oooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	ooooooooooooo•oooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	ooooooooooooo•oooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	ooooooooooooo•oooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	ooooooooooooo•oooooooo
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

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.

Bisulfite sequence information							Show options
No.	order	exclude unselected	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)	oooooooooooooo•oooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooo•oooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooo•oooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooo•oooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooo•oooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooo•oooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooo•oooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo•oooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo•oooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooo•oooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	ooo•oooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooo•oooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooo•oooooooo
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							Show options
No.	order	exclude unselected	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)	oooooooooooooo•oooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooo•oooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooo•oooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooo•oooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooo•oooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooo•oooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooo•oooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo•oooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo•oooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooo•oooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	ooo•oooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooo•oooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooo•oooooooo
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

5.6.2. Show options 2

Optional fields will appear.

Bisulfite sequence information				Hide options			
<input checked="" type="checkbox"/> Sorting conditions (CpH, CpA, CpC, CpT) <input checked="" type="checkbox"/> Conditions to exclude low quality sequences							
<input checked="" type="radio"/> user specified order <input type="radio"/> number of methylated CpGs <input type="radio"/> number of converted CpHs <input type="radio"/> percent converted CpHs <input type="radio"/> number of mismatches <input type="radio"/> percent identity <input type="radio"/> sequence name <input type="radio"/> Lower limit of percent converted CpHs				Upper limit of unconverted CpHs : 5 Lower limit of percent converted CpHs : 95.0 Upper limit of alignment mismatches : 10 Lower limit of percent identity : 90.0			
<input checked="" type="radio"/> ascending order <input type="radio"/> descending order				Reset with new parameters			
Reset Review Show figure Download figure		Download methylation status data		Download alignment data			
No	order	exclude mismatch	Sequence name	mismatch (gap) alignment length (% identity)	Mc CpG	unconverted (%) converted	Methylation pattern (or reason for the exclusion)
1	1	<input checked="" type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2 / 131 (98.5)	oooooooooooooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0 / 131 (100.0)	oooooooooooooooooooooooo
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	176 (176) / 536 (55.0)	13	46 / 93 (49.4)	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 Review Show figure		Download figure		Download methylation status data		Download alignment data	

5.6.3. Hide options

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

Bisulfite sequence information

Hide options

Sorting conditions		Conditions to exclude low quality sequences					
<input checked="" type="radio"/> user specified order	<input type="radio"/> number of methylated CpGs	Upper limit of unconverted CpHs	: 5				
<input type="radio"/> number of unconverted CpHs	<input type="radio"/> percent converted CpHs	Lower limit of percent converted CpHs	: 95.0				
<input type="radio"/> number of mismatches	<input type="radio"/> percent identity	Upper limit of alignment mismatches	: 10				
<input type="radio"/> sequence name		Lower limit of percent identity	: 90.0				
<input checked="" type="radio"/> ascending order	<input type="radio"/> descending order	Reset with new parameters					
Reset Renew Show figure Download figure		Download methylation status data Download alignment data					
No.	order	exclude mismatches >=	Sequence name	mismatch (gap) alignment length (% identity)	Me- CpG	unconverted (% converted)	Methylation pattern (or reason for exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooo•ooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooo•ooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooo•ooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooo•ooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooo•ooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooo•ooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooo•ooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo•ooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo•ooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooo•ooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oooooooooooooo•ooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooo•ooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooo•ooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.1)	1	22/112 (80.1)	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 (48.2)	unconverted, % converted
Reset Renew Show figure Download figure		Download methylation status data Download alignment data					

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 unconvertions
 - ✓ unconverted CpHs (CpA, CpC, CpT)
 - percent conversion
 - ✓ percent of converted CpHs / total CpHs
 - number of mismatches
 - percent identity
 - sequence name
 - ascending order
 - descending order

5.6.5. Change the order of bisulfite sequences 2

The change is reflected.

Bisulfite sequence information [Hide options](#)

<input checked="" type="checkbox"/> Sorting conditions (CpH, CpA, CpC, CpT)	<input checked="" type="checkbox"/> Conditions to exclude low quality sequences
<input type="radio"/> user specified order	<input type="radio"/> number of methylated CpGs
<input type="radio"/> number of unconverted CpHs	<input type="radio"/> percent converted CpHs
<input type="radio"/> number of mismatches	<input type="radio"/> percent identity
<input type="radio"/> sequence name	<input type="radio"/> ascending order
	<input type="radio"/> descending order

[Reset with new parameters](#)

[Reset](#) [Review](#) [Show figure](#) [Download figure](#) [Download methylation status data](#) [Download alignment data](#)

No.	order	exclude <input checked="" type="checkbox"/> selected all	Sequence name	mismatch / length (% identity)	Mg- unconverted (% converted)	Methylation pattern (or reason for the exclusion edit)
1	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6 / 2131 (98.5)	oooooooooooooooooooo
2	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6 / 0 / 131 (100.0)	oooooooooooooooooooo
3	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8 / 0 / 131 (100.0)	oooooooooooooooooooo
4	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9 / 0 / 130 (100.0)	oooooooooooooooooooo
5	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12 / 0 / 131 (100.0)	oooooooooooooooooooo
6	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15 / 1 / 131 (99.2)	oooooooooooooooooooo
7	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16 / 0 / 131 (100.0)	oooooooooooooooooooo
8	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18 / 0 / 130 (100.0)	oooooooooooooooooooo
9	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18 / 0 / 130 (100.0)	oooooooooooooooooooo
10	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18 / 1 / 131 (99.2)	oooooooooooooooooooo
11	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18 / 2 / 131 (98.5)	ooo...oooooooooooo
12	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19 / 0 / 131 (99.2)	oooooooooooooooooooo
13	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19 / 1 / 131 (99.2)	oooooooooooooooooooo
14	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1 / 22 / 12 (80.0)	mismatch, identity, unconverted, % converted
15	<input checked="" type="checkbox"/>	<input 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"/>	<input type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18 / 66 / 130 (49.2)	unconverted, % converted

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 CpHs (CpA, CpC and CpT)
- Lower limit of percent conversion
 - ✓ 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

5.6.7. Conditions to exclude bisulfite sequences 2

The change is reflected.

Bisulfite sequence information							<input type="checkbox"/> Hide options
Sorting conditions (CpH, CpA, CpC, CpT)			<input type="checkbox"/> Conditions to exclude low quality sequences				
<input checked="" type="radio"/> user specified order	<input type="radio"/> number of methylated CpGs	Upper limit of unconverted CpHs : 1					
<input type="radio"/> number of unconverted CpHs	<input type="radio"/> percent converted CpHs	Lower limit of percent converted CpHs : 99					
<input type="radio"/> number of mismatches	<input type="radio"/> percent identity	Upper limit of alignment mismatches : 1					
<input type="radio"/> sequence name		Lower limit of percent identity : 99					
<input checked="" type="radio"/> ascending order	<input type="radio"/> descending order	Reset with new parameters					
Reset	Review	Show figure	Download figure	Download methylation status data	Download alignment data		
No.	order	exclude MATERIAL	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion ?)
1	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooo.....ooooo
2	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooo.....ooooo
3	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	*****ooooooo.....ooooo
4	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	*****ooooooo.....ooooo
5	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	*****ooooooo.....ooooo
6	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	*****ooooooo.....ooooo
7	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	*****ooooooo.....ooooo
8	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	*****ooooooo.....ooooo
9	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	*****ooooooo.....ooooo
10	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	*****ooooooo.....ooooo
11	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	*****ooooooo.....ooooo
12	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	*****ooooooo.....ooooo
13	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	*****ooooooo.....ooooo
14	<input checked="" type="radio"/>	<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="radio"/>	<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="radio"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

Bisulfite sequence information							<input type="checkbox"/> Hide options
Sorting conditions (CpH, CpA, CpC, CpT)			<input type="checkbox"/> Conditions to exclude low quality sequences				
<input checked="" type="radio"/> user specified order	<input type="radio"/> number of methylated CpGs	Upper limit of unconverted CpHs : 1					
<input type="radio"/> number of unconverted CpHs	<input type="radio"/> percent converted CpHs	Lower limit of percent converted CpHs : 99.0					
<input type="radio"/> number of mismatches	<input type="radio"/> percent identity	Upper limit of alignment mismatches : 1					
<input type="radio"/> sequence name		Lower limit of percent identity : 99.0					
<input checked="" type="radio"/> ascending order	<input type="radio"/> descending order	Reset with new parameters					
Reset	Review	Show figure	Download figure	Download methylation status data	Download alignment data		
No.	order	exclude MATERIAL	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion ?)
1	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	ooooooooooooo.....ooooo
2	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	ooooooooooooo.....ooooo
3	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	*****ooooooo.....ooooo
4	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	*****ooooooo.....ooooo
5	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	*****ooooooo.....ooooo
6	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	*****ooooooo.....ooooo
7	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	*****ooooooo.....ooooo
8	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	*****ooooooo.....ooooo
9	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	*****ooooooo.....ooooo
10	<input checked="" type="radio"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
11	<input checked="" type="radio"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	unconverted, % converted
12	<input checked="" type="radio"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	mismatch
13	<input checked="" type="radio"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
14	<input checked="" type="radio"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	mismatch
15	<input checked="" type="radio"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	mismatch, unconverted, % converted
16	<input checked="" type="radio"/>	<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.

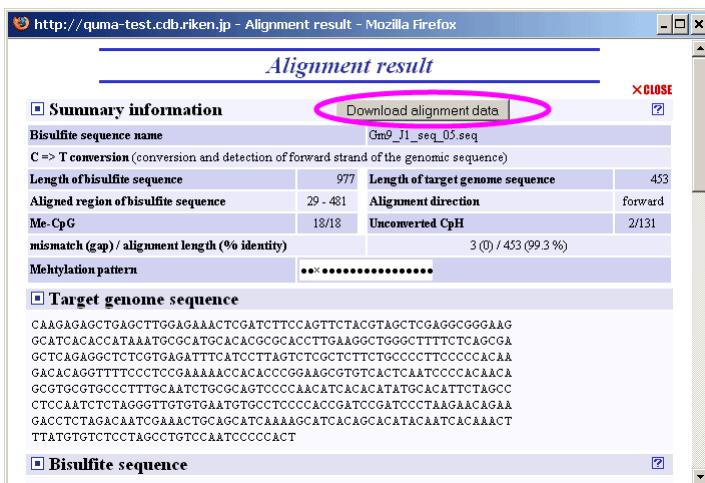
D) Bisulfite alignment

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



5.7.2. Download alignment data

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



5.7.3. Alignment data

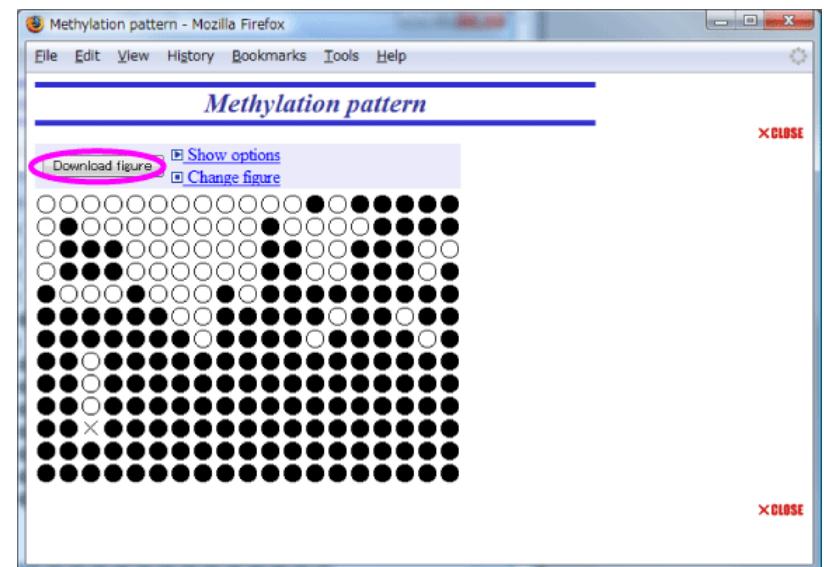
Downloaded alignment data file can be opened byTextEdit (Mac), Notepad (Win) or other text editors.



5.8. Figure page

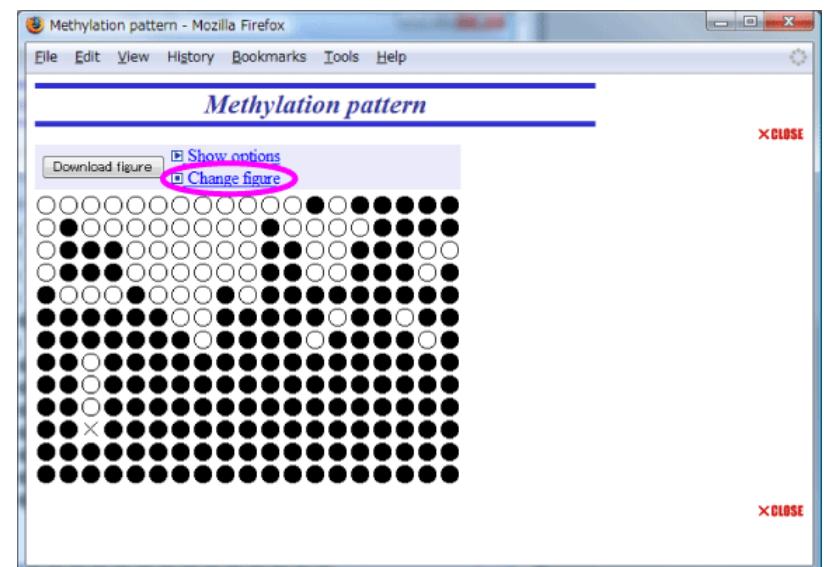
5.8.1. Download methylation pattern figure

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



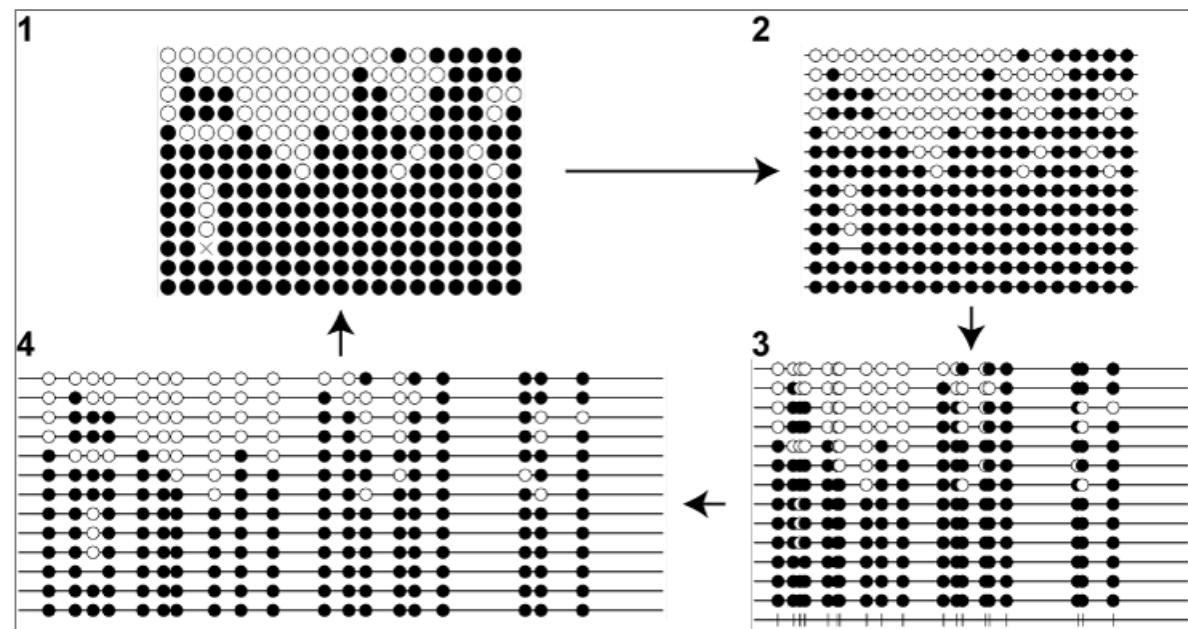
5.8.2. Change methylation pattern figure 1

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



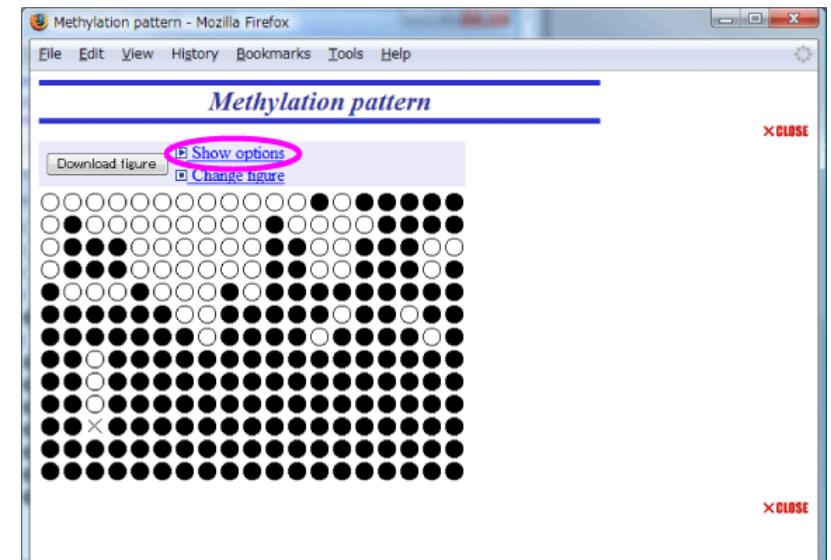
5.8.3. Change methylation pattern figure 2

Methylation pattern figures are switched one after the other.



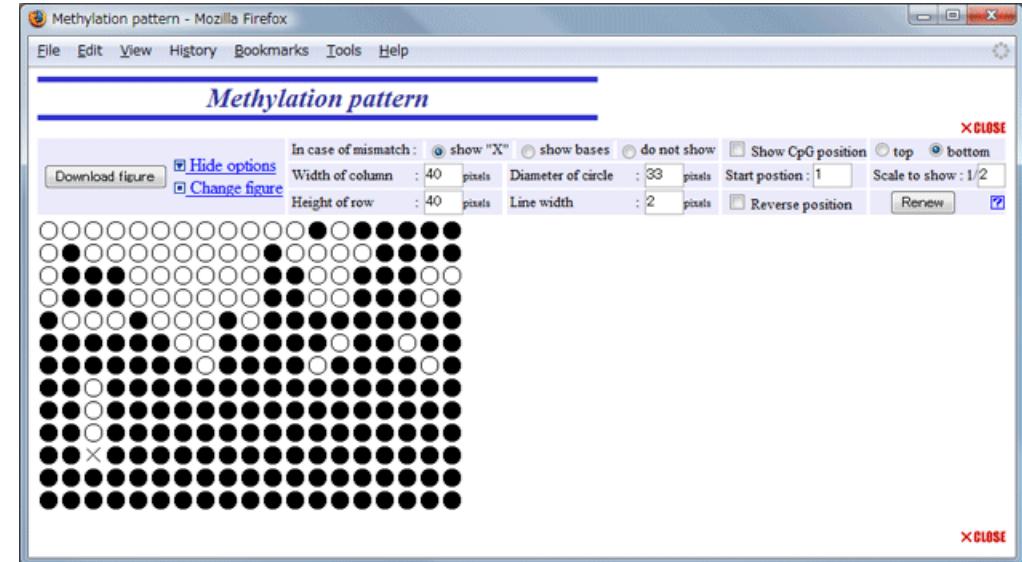
5.8.4. Show options

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



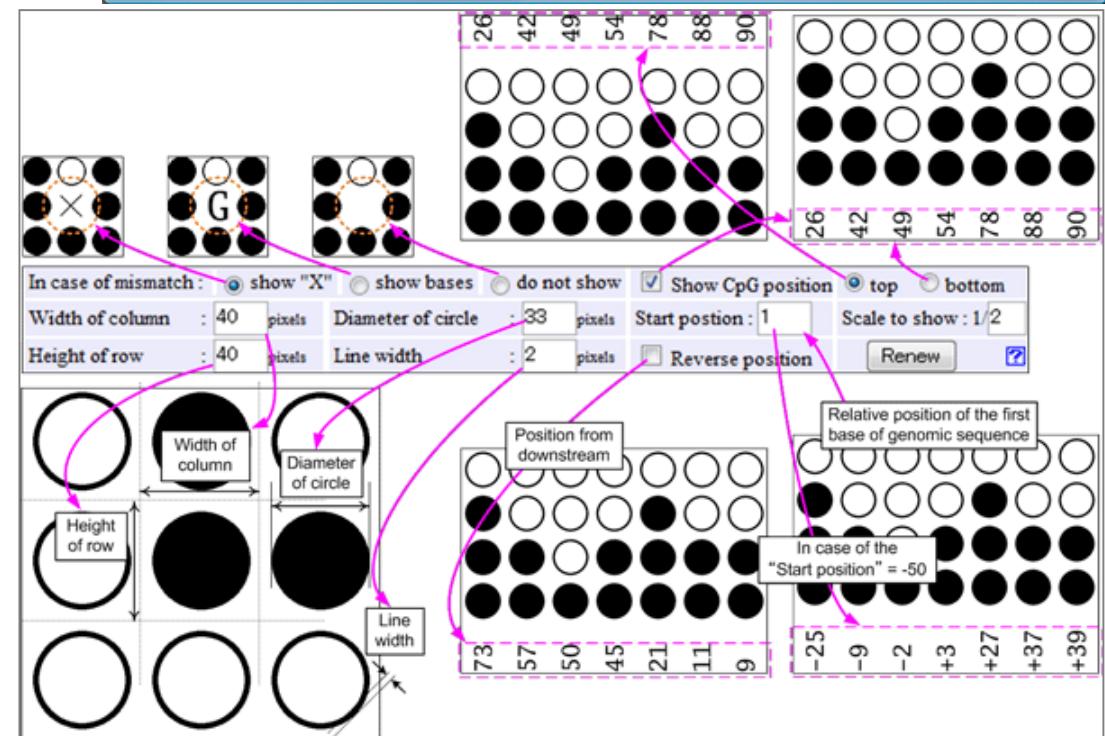
5.8.5. Figure 1

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.



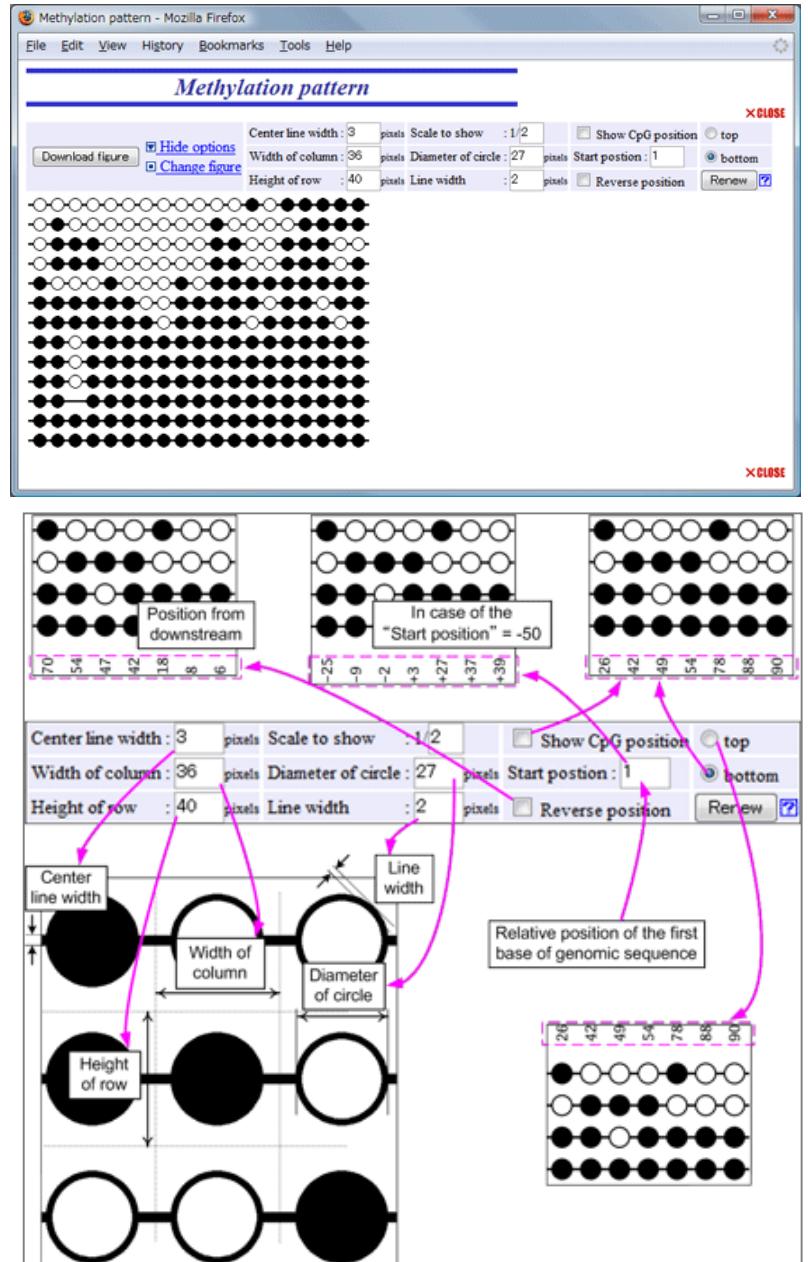
5.8.7. Figure 2

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.

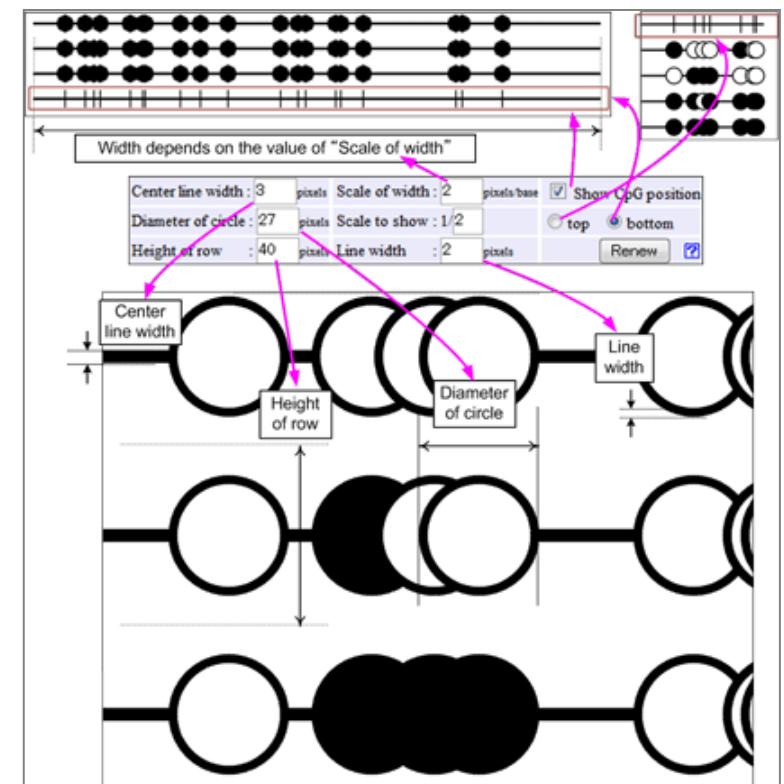
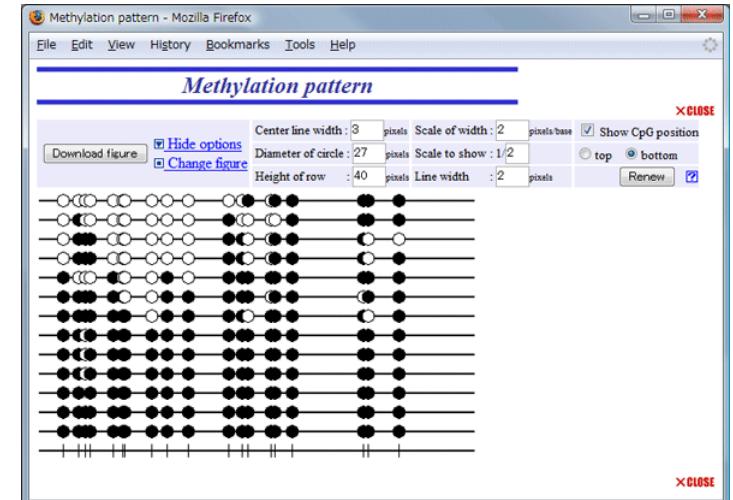


5.8.9. Figure 3

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.

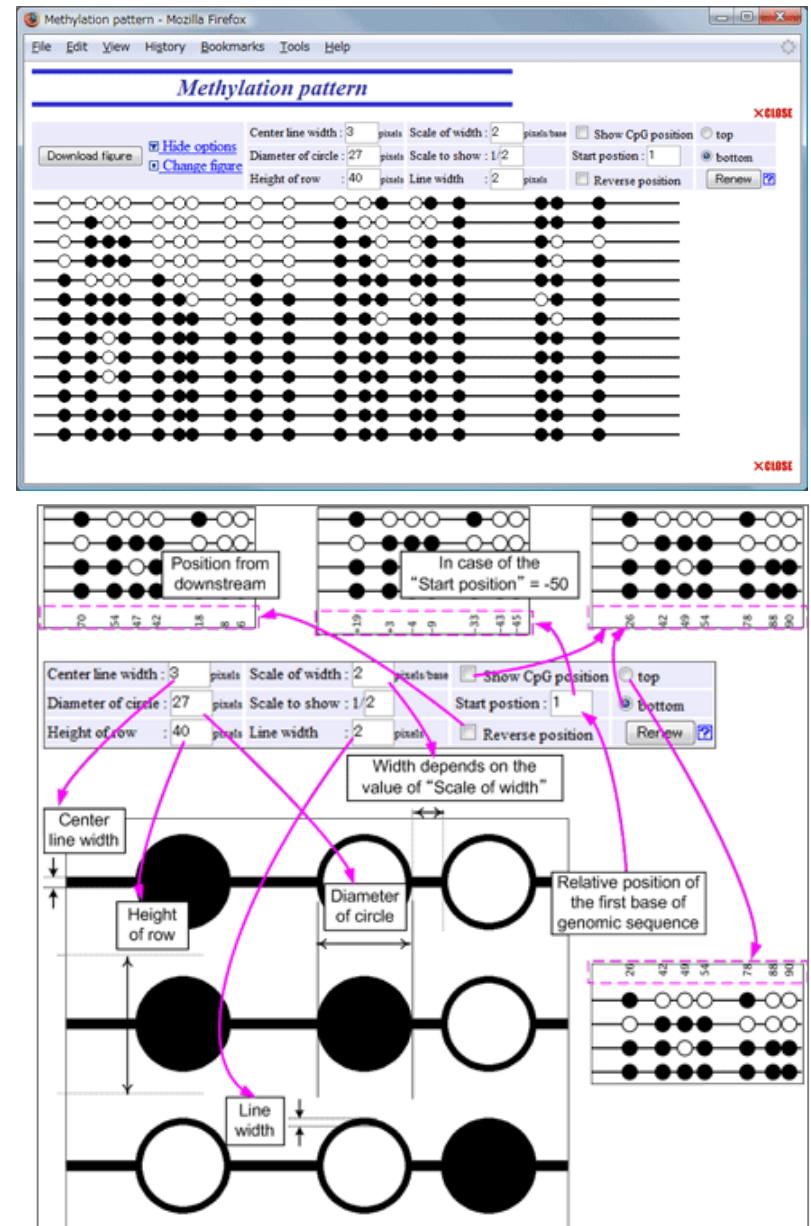


5.8.11. Figure 4

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.

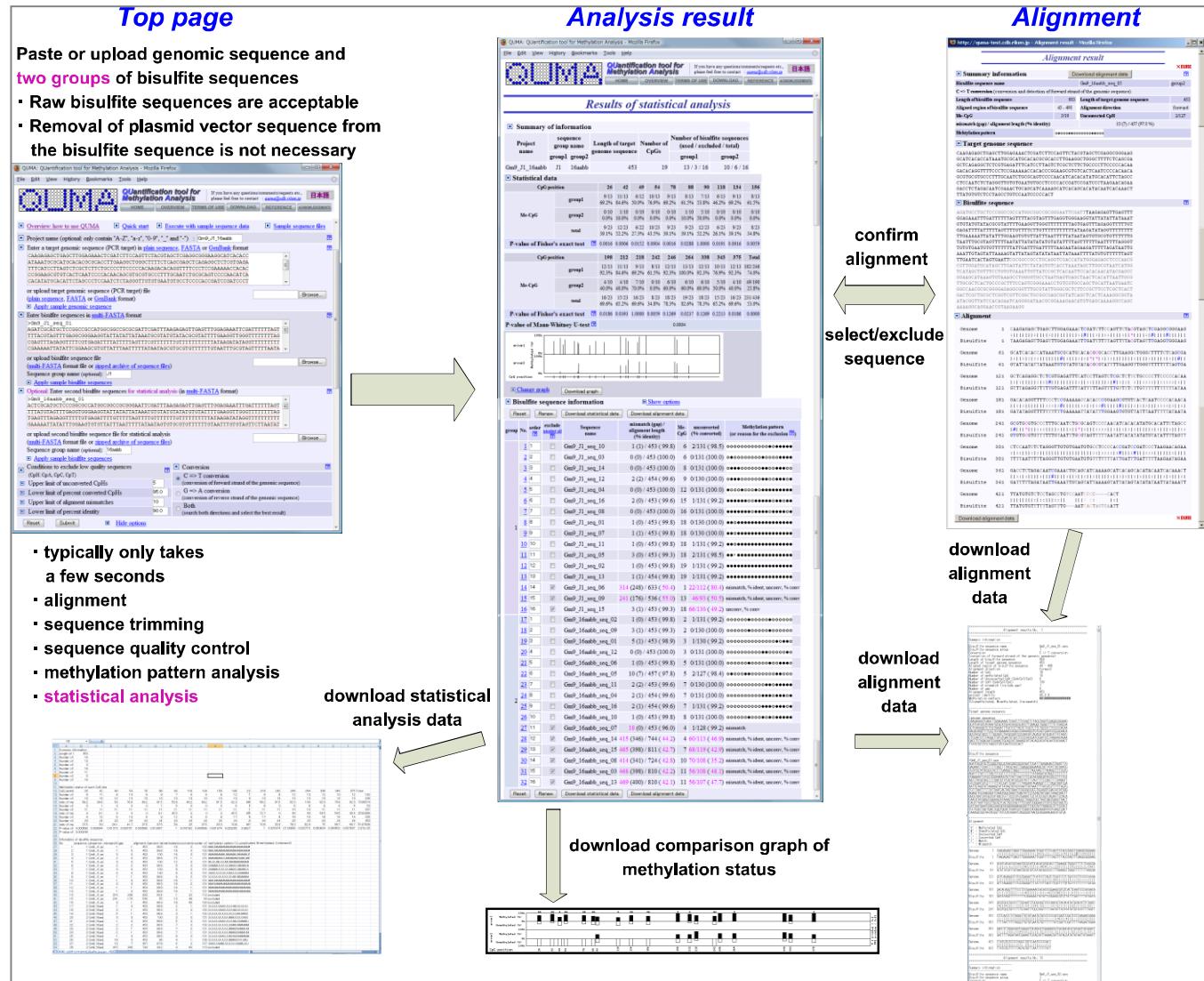


6. Statistical analysis mode

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](#).



6.2. Top page

6.2.1. Show options

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

The screenshot shows the QUMA homepage. At the top, there is a navigation bar with links for File, Edit, View, History, Bookmarks, Tools, and Help. Below the navigation bar is a logo consisting of the letters 'QUMA' in blue and white, followed by the text 'Quantification tool for Methylation Analysis'. A message in Japanese says 'If you have any questions/comments/requests etc., please feel free to contact : quma@cb.riken.jp' and '日本語'. Below the logo, the main title 'QUantification tool for Methylation Analysis' is displayed in large blue letters. A sub-section title 'You can easily align, visualize and quantify bisulfite sequence data for CpG methylation analysis' follows. There are several input fields and checkboxes:

- 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.

Buttons: Reset, Submit, Show options (highlighted with a red circle).

The screenshot shows the QUMA interface with the 'Show options' section expanded. At the top, there is a navigation bar with links for File, Edit, View, History, Bookmarks, Tools, and Help. Below the navigation bar is a logo consisting of the letters 'QUMA' in blue and white, followed by the text 'Quantification tool for Methylation Analysis'. A message in Japanese says 'If you have any questions/comments/requests etc., please feel free to contact : quma@cb.riken.jp' and '日本語'. Below the logo, the main title 'QUantification tool for Methylation Analysis' is displayed in large blue letters. A sub-section title 'HOME' is visible. There are several input fields and checkboxes:

- 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)
or upload second bisulfite sequence file for statistical analysis ([multi-FASTA](#) format file or [zipped archive of sequence files](#))
Sequence group name (optional):
 Apply sample bisulfite sequences

Conditions to exclude low quality sequences
(CpI: CpA, CpC, CpT)
 Upper limit of unconverted CpHs:
 Lower limit of percent converted CpHs:
 Upper limit of alignment mismatches:
 Lower limit of percent identity:

Conversion
 C => T conversion
(conversion of forward strand of the genomic sequence)
 G => A conversion
(conversion of reverse strand of the genomic sequence)
 Both
(search both directions and select the best result)

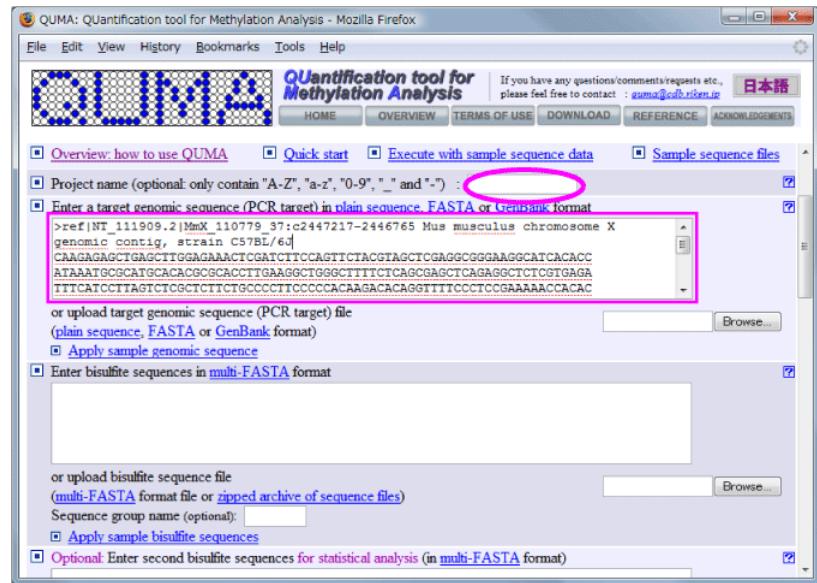
Buttons: Reset, Submit, Hide options.

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](#)".



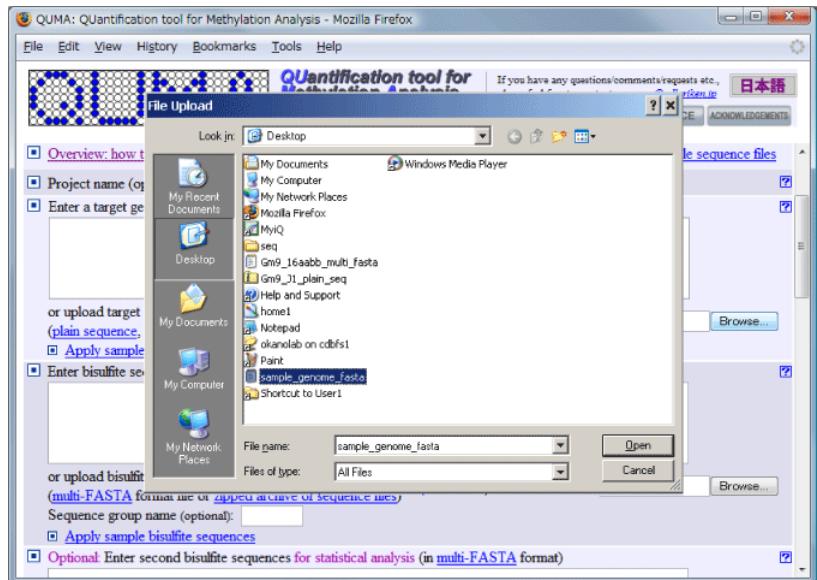
6.2.4. Genomic sequence file 1

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



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).

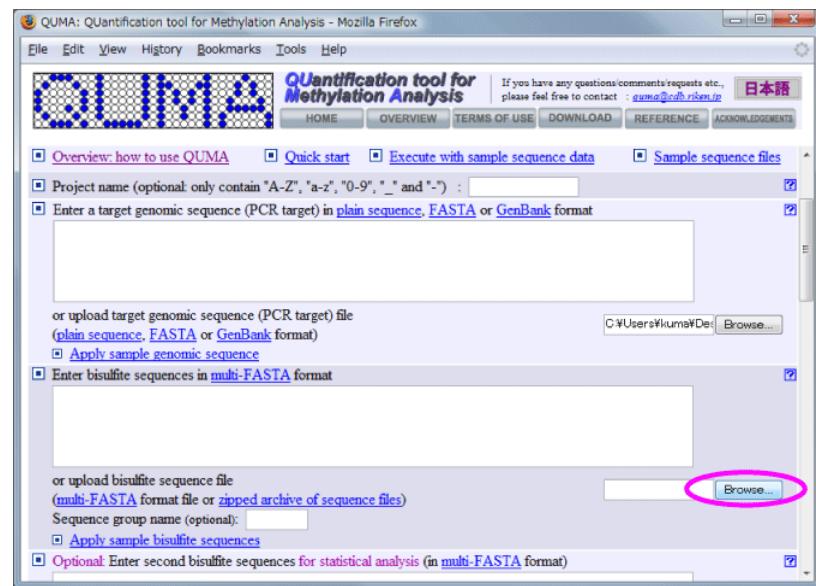
The bisulfite sequences can be input by two ways of 1) direct input and 2) upload.

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

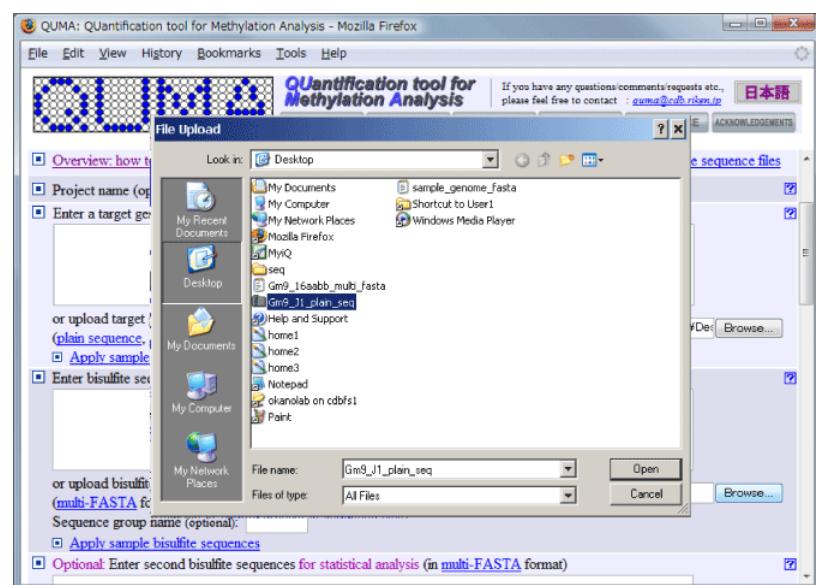
2) Or click the second button to upload a file of bisulfite sequences of first group.



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 \(Windows\)](#)".



6.2.9. Second bisulfite sequence group

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

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.



6.2.10. File of second bisulfite sequence group 1

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



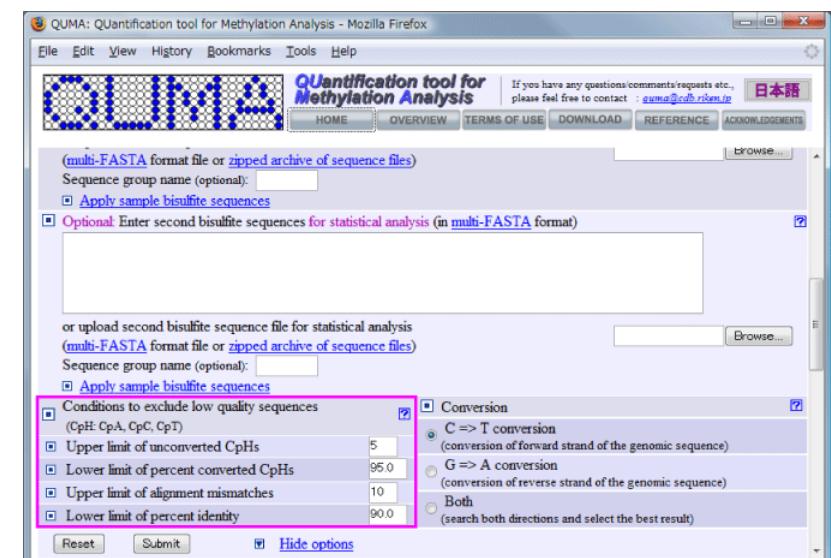
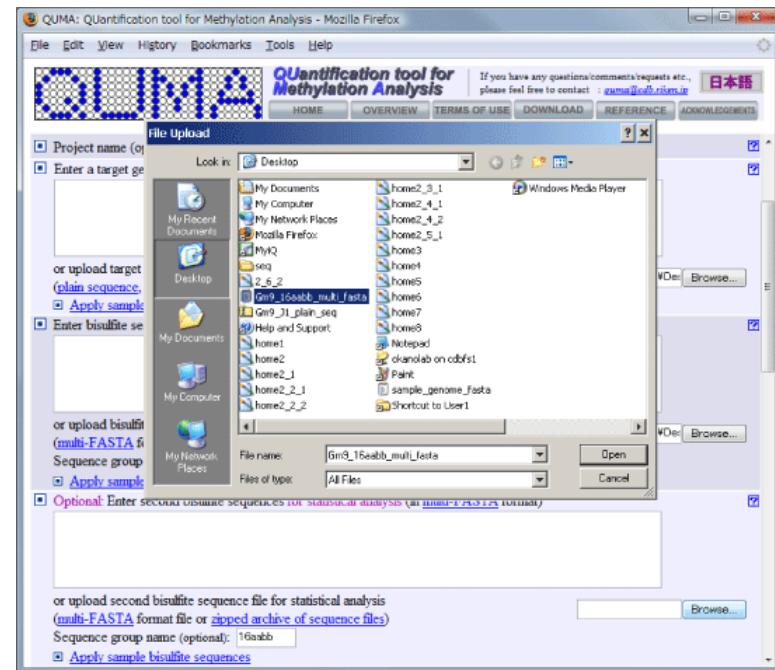
6.2.11. File of second bisulfite sequence group 2

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.

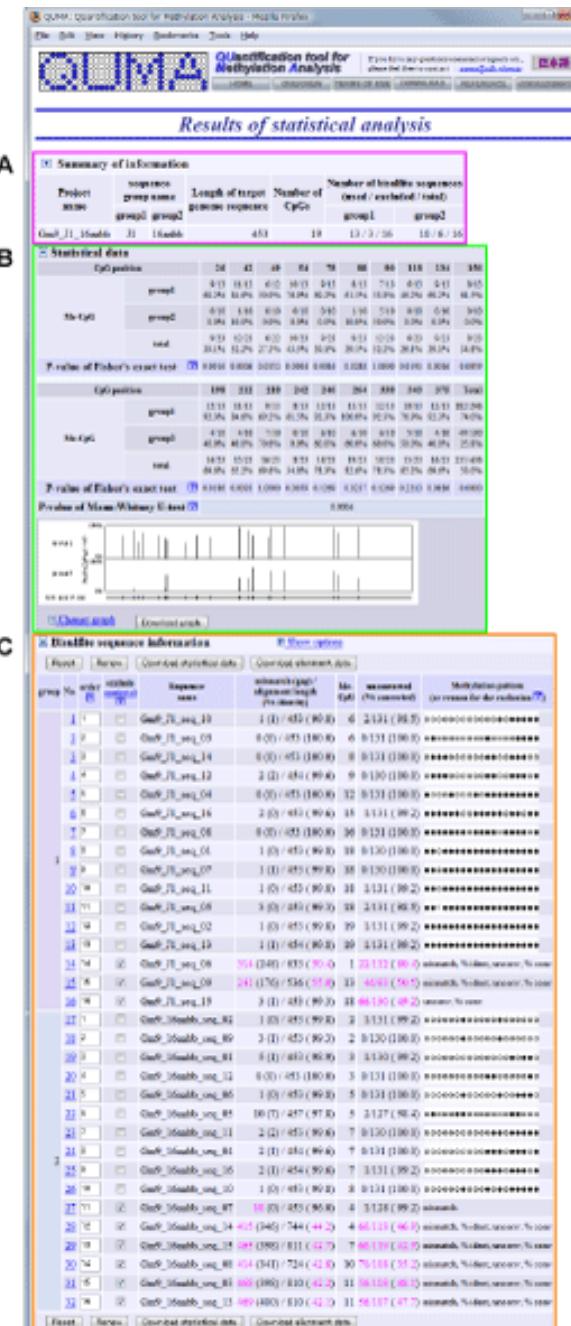
The screenshots show the QUMA web interface for 'Quantification tool for Methylation Analysis'. The top screenshot displays the 'Conversion' section, which includes options for 'C => T conversion' (selected), 'G => A conversion', and 'Both'. The bottom screenshot highlights the 'Submit' button with a pink circle, indicating where the user should click to start the analysis.

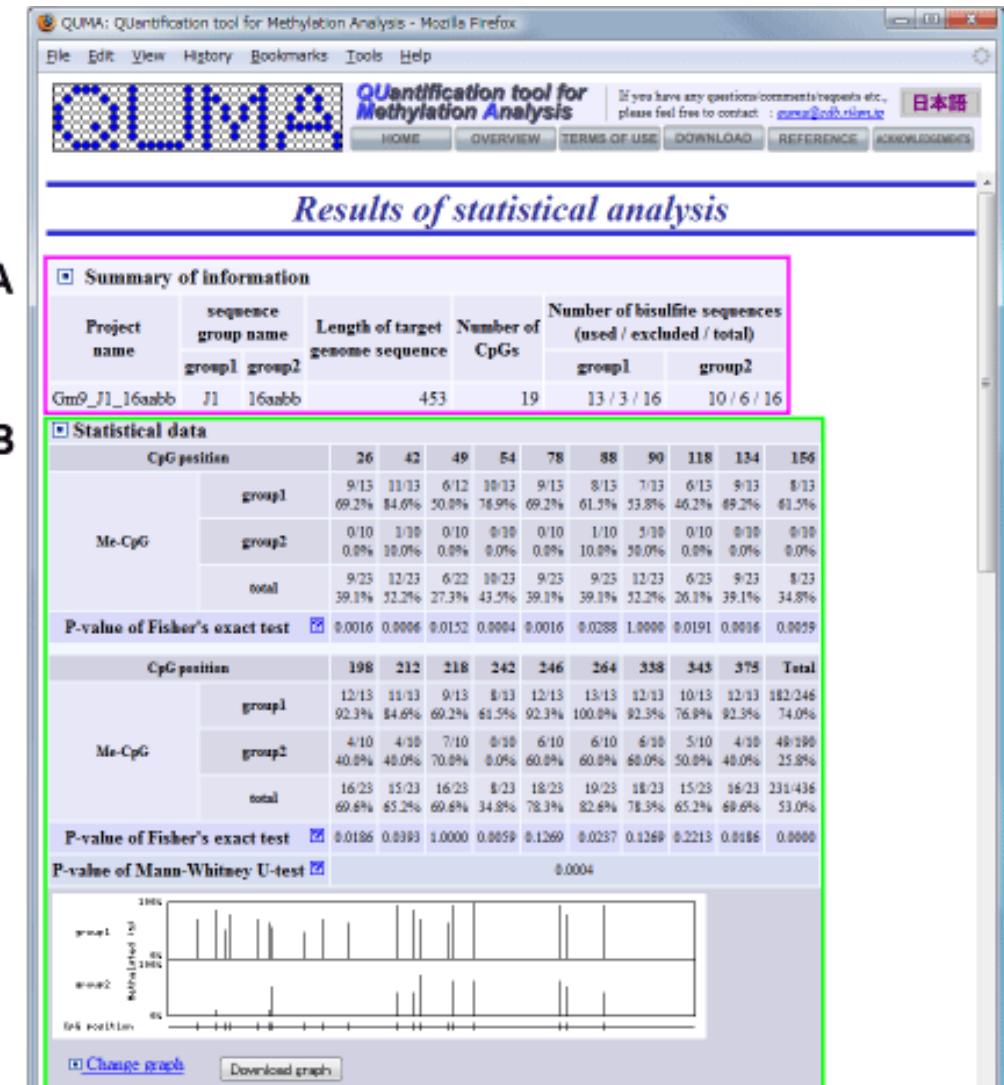
6.3. Statistical analysis result page

6.3.1. Overview of statistical analysis result page 1

Statistical analysis result page consists of three sections.

- A) Summary of information
 - B) Statistical data
 - C) 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.

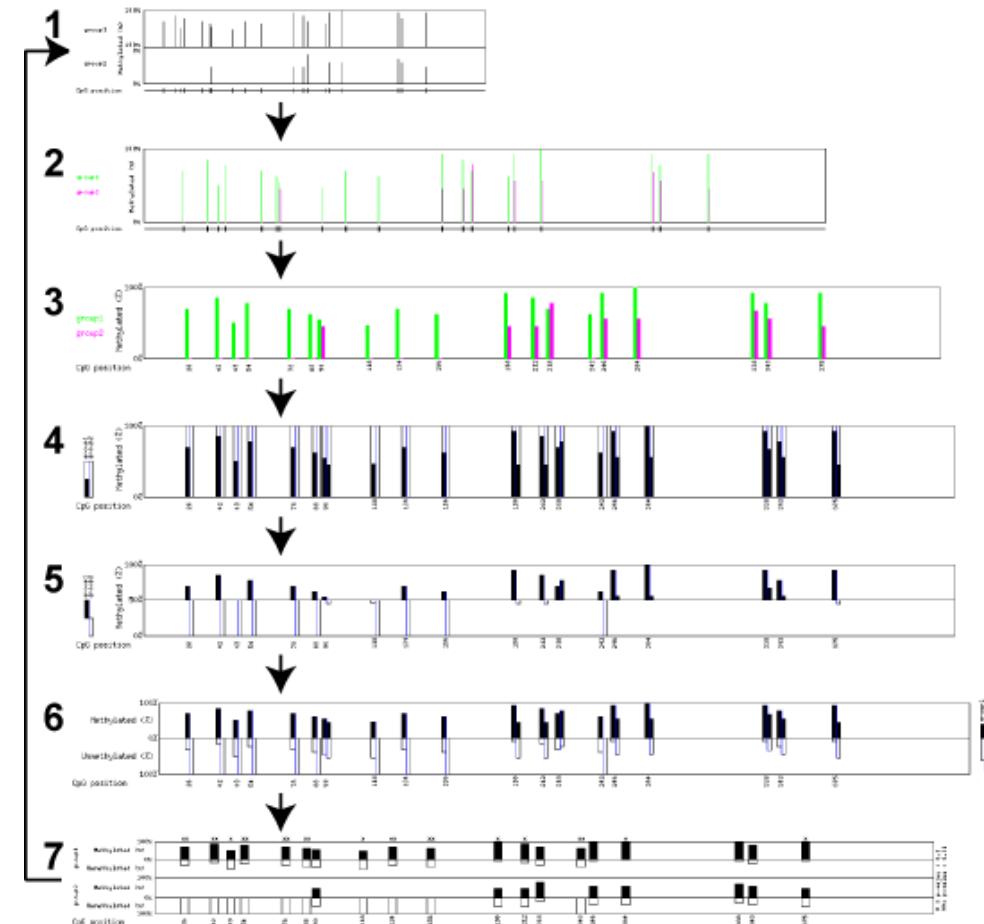
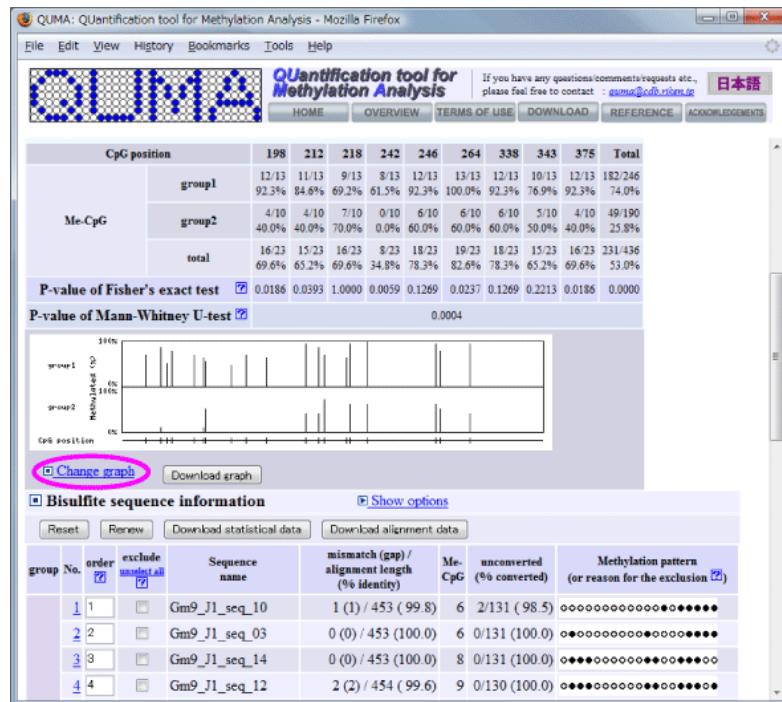
Mann-Whitney U-test: The statistical significance between two groups of the entire set of CpG sites is evaluated with the Mann-Whitney U-test (also called the Wilcoxon rank-sum test) that is non-parametric statistical significance test for two distributed samples. See "[9.2. Mann-Whitney U-test](#)"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

6.3.3. Change methylation status figure 1

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

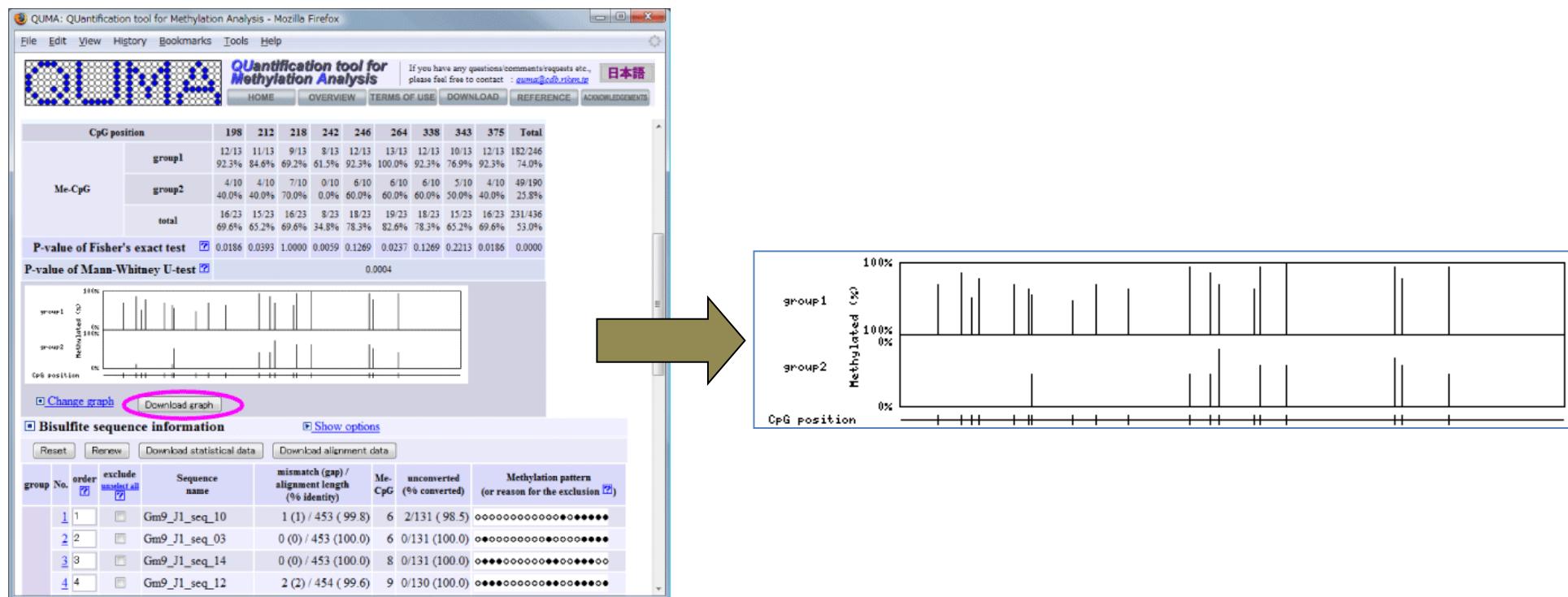


6.3.4. Change methylation status figure 2

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.



C

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 CpHs (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).

Bisulfite sequence information

Show options

Reset Renew Download statistical data Dow 1. Alignment 2. 3. 4.

group	No.	order	exclude methyl all	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	1			Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
	2			Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	ooooooooooooooooooo
	3			Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	ooooooooooooooooooo
	4			Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	ooooooooooooooooooo
	5			Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	ooooooooooooooooooo
	6			Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	ooooooooooooooooooo
	7			Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	ooooooooooooooooooo
	8			Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooooooooo
	9			Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooooooooo
	10			Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	ooooooooooooooooooo
	11	11		Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	ooxxoooooooooooo
	12	12		Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	ooooooooooooooooooo
	13	13		Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	ooooooooooooooooooo
	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		Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	ooooooooooooooooooo
	18	2		Gm9_16aabb_seq_09	3 (1) / 453 (99.3)	2	0/130 (100.0)	ooooooooooooooooooo
	19	3		Gm9_16aabb_seq_01	5 (1) / 453 (98.9)	3	1/130 (99.2)	ooooooooooooooooooo
	20	4		Gm9_16aabb_seq_12	0 (0) / 453 (100.0)	3	0/131 (100.0)	ooooooooooooooooooo
	21	5		Gm9_16aabb_seq_06	1 (0) / 453 (99.8)	5	0/131 (100.0)	ooooooooooooooooooo
	22	6		Gm9_16aabb_seq_05	10 (7) / 457 (97.8)	5	2/127 (98.4)	ooooooooooooooooooo
	23	7		Gm9_16aabb_seq_11	2 (2) / 453 (99.6)	7	0/130 (100.0)	ooooooooooooooooooo
	24	8		Gm9_16aabb_seq_04	2 (1) / 454 (99.6)	7	0/131 (100.0)	ooooooooooooooooooo
	25	9		Gm9_16aabb_seq_16	2 (1) / 454 (99.6)	7	1/131 (99.2)	ooooooooooooooooooo
	26	10		Gm9_16aabb_seq_10	1 (0) / 453 (99.8)	8	0/131 (100.0)	ooooooooooooooooooo
	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

Reset Renew Download statistical data Download alignment data

- **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.

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.

group	order	exclude <input type="checkbox"/>	Sequence name	mismatch (gap) / alignment length (%) identity	Me-CpG (%) converted	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6 / 131 (98.5)	oooooooooooooooooooo
2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6 / 131 (100.0)	ooooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8 / 131 (100.0)	ooooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9 / 130 (100.0)	ooooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12 / 131 (100.0)	ooooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15 / 131 (99.2)	ooooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16 / 131 (100.0)	ooooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18 / 130 (100.0)	ooooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18 / 130 (100.0)	ooooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18 / 131 (99.2)	ooooooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18 / 131 (98.5)	ooooooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19 / 131 (99.2)	ooooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19 / 131 (99.2)	ooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1 / 22/12 (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
17	1	<input type="checkbox"/>	Gm9_16aabbb_seq_02	1 (0) / 453 (99.8)	2 / 131 (99.2)	ooooooooooooooooooo
18	2	<input type="checkbox"/>	Gm9_16aabbb_seq_09	3 (1) / 453 (99.3)	2 / 0/130 (100.0)	ooooooooooooooooooo
19	3	<input type="checkbox"/>	Gm9_16aabbb_seq_01	5 (1) / 453 (98.9)	3 / 1/130 (99.2)	ooooooooooooooooooo
20	4	<input type="checkbox"/>	Gm9_16aabbb_seq_12	0 (0) / 453 (100.0)	3 / 0/131 (100.0)	ooooooooooooooooooo
21	5	<input type="checkbox"/>	Gm9_16aabbb_seq_06	1 (0) / 453 (99.8)	5 / 0/131 (100.0)	ooooooooooooooooooo
22	6	<input type="checkbox"/>	Gm9_16aabbb_seq_11	2 (2) / 453 (99.6)	7 / 0/130 (100.0)	ooooooooooooooooooo
23	7	<input type="checkbox"/>	Gm9_16aabbb_seq_04	2 (1) / 454 (99.6)	7 / 0/131 (100.0)	ooooooooooooooooooo
24	8	<input type="checkbox"/>	Gm9_16aabbb_seq_16	2 (1) / 454 (99.6)	7 / 1/131 (99.2)	ooooooooooooooooooo
25	9	<input type="checkbox"/>	Gm9_16aabbb_seq_10	1 (0) / 453 (99.8)	8 / 0/131 (100.0)	ooooooooooooooooooo
26	10	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_07	18 (0) / 453 (96.0)	4 / 1/128 (99.2)	mismatch
27	11	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_14	415 (346) / 744 (44.2)	4 / 60/113 (46.9)	mismatch, % ident, unconv, % conv
28	12	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_05	10 (7) / 457 (97.8)	5 / 2/127 (98.4)	mismatch
29	13	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_15	465 (398) / 811 (42.7)	7 / 68/119 (42.9)	mismatch, % ident, unconv, % conv
30	14	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_08	414 (341) / 724 (42.8)	10 / 70/108 (35.2)	mismatch, % ident, unconv, % conv
31	15	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_03	468 (398) / 810 (42.2)	11 / 56/108 (48.1)	mismatch, % ident, unconv, % conv
32	16	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_13	469 (400) / 810 (42.1)	11 / 56/107 (47.7)	mismatch, % ident, unconv, % conv

group	order	exclude <input type="checkbox"/>	Sequence name	mismatch (gap) / alignment length (%) identity	Me-CpG (%) converted	Methylation pattern (or reason for the exclusion)
1	1	<input type="checkbox"/>	Gm9_J1_seq_12	1 (1) / 453 (99.8)	1 / 1/131 (99.4)	ooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1 / 22/12 (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
17	1	<input type="checkbox"/>	Gm9_16aabbb_seq_02	1 (0) / 453 (99.8)	2 / 1/131 (99.2)	ooooooooooooooooooo
18	2	<input type="checkbox"/>	Gm9_16aabbb_seq_09	3 (1) / 453 (99.3)	2 / 0/130 (100.0)	ooooooooooooooooooo
19	3	<input type="checkbox"/>	Gm9_16aabbb_seq_01	5 (1) / 453 (98.9)	3 / 1/130 (99.2)	ooooooooooooooooooo
20	4	<input type="checkbox"/>	Gm9_16aabbb_seq_12	0 (0) / 453 (100.0)	3 / 0/131 (100.0)	ooooooooooooooooooo
21	5	<input type="checkbox"/>	Gm9_16aabbb_seq_06	1 (0) / 453 (99.8)	5 / 0/131 (100.0)	ooooooooooooooooooo
22	6	<input type="checkbox"/>	Gm9_16aabbb_seq_11	2 (2) / 453 (99.6)	7 / 0/130 (100.0)	ooooooooooooooooooo
23	7	<input type="checkbox"/>	Gm9_16aabbb_seq_04	2 (1) / 454 (99.6)	7 / 0/131 (100.0)	ooooooooooooooooooo
24	8	<input type="checkbox"/>	Gm9_16aabbb_seq_16	2 (1) / 454 (99.6)	7 / 1/131 (99.2)	ooooooooooooooooooo
25	9	<input type="checkbox"/>	Gm9_16aabbb_seq_10	1 (0) / 453 (99.8)	8 / 0/131 (100.0)	ooooooooooooooooooo
26	10	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_07	18 (0) / 453 (96.0)	4 / 1/128 (99.2)	mismatch
27	11	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_14	415 (346) / 744 (44.2)	4 / 60/113 (46.9)	mismatch, % ident, unconv, % conv
28	12	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_05	10 (7) / 457 (97.8)	5 / 2/127 (98.4)	mismatch
29	13	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_15	465 (398) / 811 (42.7)	7 / 68/119 (42.9)	mismatch, % ident, unconv, % conv
30	14	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_08	414 (341) / 724 (42.8)	10 / 70/108 (35.2)	mismatch, % ident, unconv, % conv
31	15	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_03	468 (398) / 810 (42.2)	11 / 56/108 (48.1)	mismatch, % ident, unconv, % conv
32	16	<input checked="" type="checkbox"/>	Gm9_16aabbb_seq_13	469 (400) / 810 (42.1)	11 / 56/107 (47.7)	mismatch, % ident, unconv, % conv

6.3.9. Include/exclude bisulfite sequence 2

The change is reflected.

Quantification tool for Methylation Analysis						
				HOME OVERVIEW TERMS OF USE DOWNLOAD REFERENCE ACKNOWLEDGEMENTS		
13	<input checked="" type="checkbox"/>	Mm9_J1_seq_13	1 (1) / 453 (99.8)	19	1/131 (99.2)	*****
14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, %ident, unconv, %conv
15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, %ident, unconv, %conv
16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, %conv
17	<input type="checkbox"/>	Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	oooooooooooooooooooooo
18	<input type="checkbox"/>	Gm9_16aabb_seq_09	3 (1) / 453 (99.3)	2	0/130 (100.0)	oooooooooooooooooooooo
19	<input type="checkbox"/>	Gm9_16aabb_seq_01	5 (1) / 453 (98.9)	3	1/130 (99.2)	oooooooooooooooooooooo
20	<input type="checkbox"/>	Gm9_16aabb_seq_12	0 (0) / 453 (100.0)	3	0/131 (100.0)	oooooooooooooooooooooo
21	<input type="checkbox"/>	Gm9_16aabb_seq_06	1 (0) / 453 (99.8)	5	0/131 (100.0)	oooooooooooooooooooooo
22	<input type="checkbox"/>	Gm9_16aabb_seq_11	2 (2) / 453 (99.6)	7	0/130 (100.0)	oooooooooooooooooooooo
23	<input type="checkbox"/>	Gm9_16aabb_seq_04	2 (1) / 454 (99.6)	7	0/131 (100.0)	oooooooooooooooooooooo
24	<input type="checkbox"/>	Gm9_16aabb_seq_16	2 (1) / 454 (99.6)	7	1/131 (99.2)	oooooooooooooooooooooo
25	<input type="checkbox"/>	Gm9_16aabb_seq_10	1 (0) / 453 (99.8)	8	0/131 (100.0)	oooooooooooooooooooooo
26	<input type="checkbox"/>	Gm9_16aabb_seq_07	18 (0) / 453 (96.0)	4	1/128 (99.2)	oooooooooooooooooooooo
27	<input type="checkbox"/>	Gm9_16aabb_seq_05	10 (7) / 457 (97.8)	5	2/127 (98.4)	oooooooooooooooooooooo
28	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_14	415 (346) / 744 (44.2)	4	60/113 (46.9)	mismatch, %ident, unconv, %conv
29	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_15	465 (398) / 811 (42.7)	7	68/119 (42.9)	mismatch, %ident, unconv, %conv
30	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_08	414 (341) / 724 (42.8)	10	70/108 (35.2)	mismatch, %ident, unconv, %conv
31	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_03	468 (398) / 810 (42.2)	11	56/108 (48.1)	mismatch, %ident, unconv, %conv
32	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_13	469 (400) / 810 (42.1)	11	56/107 (47.7)	mismatch, %ident, unconv, %conv

Quantification tool for Methylation Analysis						
				HOME OVERVIEW TERMS OF USE DOWNLOAD REFERENCE ACKNOWLEDGEMENTS		
<input checked="" type="checkbox"/> Bisulfite sequence information						
		<input type="radio"/>	Reset	<input type="radio"/>	Renew	<input type="radio"/>
				<input type="radio"/>	Download statistical data	<input type="radio"/>
					<input type="radio"/>	Download alignment data
group No.	order	exclude	Sequence name	mismatch (gap)/alignment length (% identity)	Me-CpG (%)	unconverted (% converted)
		<input checked="" type="checkbox"/>	sequence_id		(% converted)	(or reason for the exclusion)
1	19	<input checked="" type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)
2	12	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)
3	11	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)
4	10	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)
5	9	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)
6	8	<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	6	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)
9	5	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)
10	4	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)
11	3	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)
12	2	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)
13	1	<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)
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)
17	1	<input type="checkbox"/>	Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)

6.3.11. Change the order of bisulfite sequences 2

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

group No.	order	exclude mismatch all	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted	Methylation pattern (or reason for the exclusion)
1			Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	*****ooooooooooooo*****
2			Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	*****ooooooooooooo*****
3			Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	***ooooooooooooo*****
4			Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	*****ooooooooooooo*****
5			Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	*****ooooooooooooo*****
6			Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	*****ooooooooooooo*****
7			Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	*****ooooooooooooo*****
8			Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	*****ooooooooooooo*****
9			Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	*****ooooooooooooo*****
10			Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	*****ooooooooooooo*****
11			Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	*****ooooooooooooo*****
12			Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	*****ooooooooooooo*****
13			Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	ooooooooooooooooooooo*****
14			Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, %ident, unconv, %conv
15			Gm9_J1_seq_09	341 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, %ident, unconv, %conv
16			Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, %conv
17			Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	ooooooo*ooooo*ooooo*****

6.3.12. Download alignments data

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

group No.	order	exclude mismatch all	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted	Methylation pattern (or reason for the exclusion)
1			Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	ooooooooooooooooooooo*****
2			Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	ooooooooooooooooooooo*****
3			Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	ooooooooooooooooooooo*****
4			Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	ooooooooooooooooooooo*****
5			Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	ooooooooooooooooooooo*****
6			Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	*****ooooooooooooo*****
7			Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	*****ooooooooooooo*****
8			Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	*****ooooooooooooo*****
9			Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	*****ooooooooooooo*****
10			Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	*****ooooooooooooo*****
11			Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	***ooooooooooooo*****
12			Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	*****ooooooooooooo*****
13			Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	*****ooooooooooooo*****
14			Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, %ident, unconv, %conv
15			Gm9_J1_seq_09	341 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, %ident, unconv, %conv
16			Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, %conv
17			Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	ooooooo*ooooo*ooooo*****

6.3.13. Alignments data

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



6.3.14. Download statistical analysis data

Click "Download statistical data" button to download statistical analysis data.

The screenshot shows the QUMA software interface with the title bar 'QUMA: Quantification tool for Methylation Analysis - Mozilla Firefox'. Below the title bar is a menu bar with File, Edit, View, History, Bookmarks, Tools, and Help. There is also a language selection for Japanese. The main window displays a table titled 'Bisulfite sequence information' with several columns: group No., order, exclude sample all, Sequence name, mismatch (gap), alignment length (% identity), Me CpG, unconverted (% converted), and Methylation pattern (or reason for the exclusion). The 'Download statistical data' button is highlighted with a red circle.

6.3.15. Statistical analysis data

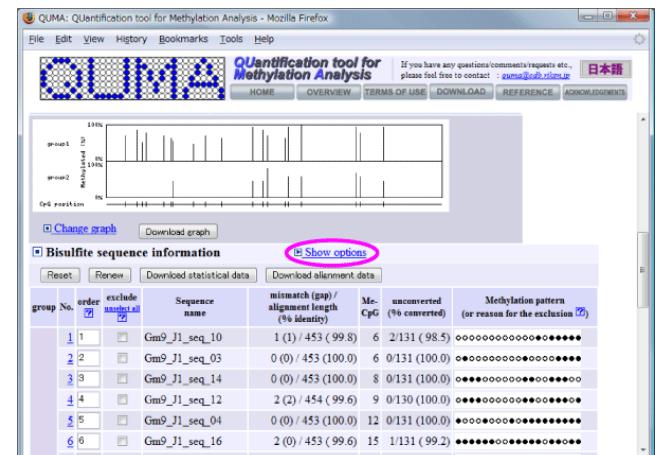
Downloaded statistical analysis data file can be opened by Microsoft Excel, [OpenOffice/StartSuite](#) or other spreadsheet software ([CSV](#) file format). See also “[10.1. How to open a CSV file](#)”.

The screenshot shows a Microsoft Excel spreadsheet with data starting from row 12. Row 12 contains headers for 'Length of 1', 'Number of 1', 'Number of 1', 'Number of 3', 'Number of 16', 'Number of 11', 'Number of 5', and 'Number of 16'. Rows 13 through 17 show data for each of these categories. Row 18 contains 'ratio of me' values for each category. Rows 19 through 21 show 'Number of' values. Row 22 contains 'P-value of' and 'Pr-value of' followed by their respective values. Rows 23 and 24 contain 'Pr-value of' and 'Pr-value of' again. Row 25 contains 'Information of bisulfite sequence' with columns for 'Nb.', 'sequence', 'sequence (mismatch)', 'alignment', 'percent of methylated', 'unconverted', 'number of', and 'methylation pattern (U unmethylated, M methylated, X mismatch)'. Rows 26 through 54 show individual sequence entries with their corresponding values for each column.

6.4. Statistical analysis result page options

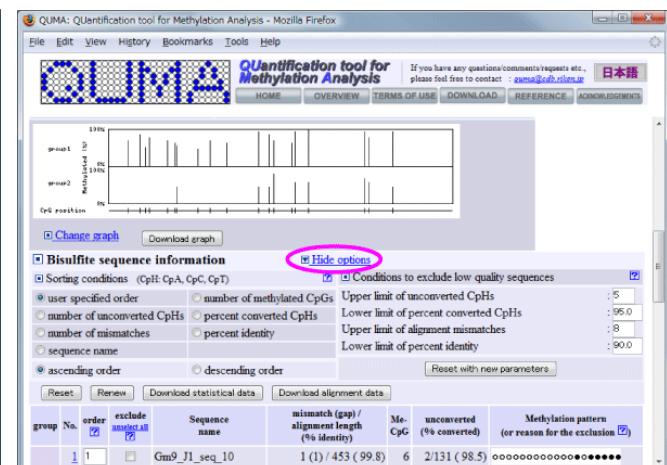
6.4.1. Show options 1

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



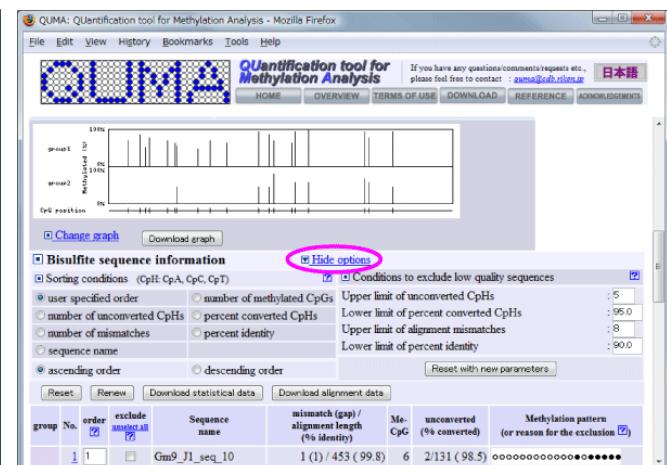
6.4.2. Show options 2

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 unconvertions
 - ✓ unconverted CpHs (CpA, CpC, CpT)
- percent conversion
 - ✓ percent of converted CpHs / total CpHs
- number of mismatches
- percent identity
- sequence name
- ascending order
- descending order

group	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)	ooooooooooooo*****
	2	2	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	ooooooooooooo*****
	3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	ooooooooooooo*****
	4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	ooooooooooooo*****
	5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	ooooooocoooo*****
	6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	ooooooooooooo*****
	7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	ooooooooooooo*****
	8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooo*****
1	9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooo*****
	10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	ooooooooooooo*****
	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)	ooooooooooooo*****
	13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	ooooooooooooo*****
	14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, % ident, unconv, % conv

group	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_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	ooooooooooooo*****
	2	2	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	ooooooooooooo*****
	3	3	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	ooooooooooooo*****
	4	4	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	ooooooooooooo*****
	5	5	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	ooooooooooooo*****
	6	6	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	ooooooooooooo*****
	7	7	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	ooooooooooooo*****
1	8	8	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooo*****
	9	9	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	ooooooooooooo*****
	10	10	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooo*****
	11	11	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 451 (99.6)	15	1/131 (99.2)	ooooooooooooo*****
	12	12	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	ooooooooooooo*****
	13	13	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	***
	14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, % conv

6.4.6. 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 CpHs (CpA, CpC and CpT)
- Lower limit of percent conversion
 - ✓ 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

6.4.7. Conditions to exclude bisulfite sequences 2

The change is reflected.

group	No.	order	exclude	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG	unconverted (%) converted)	Methylation pattern (or reason for the exclusion)
1	1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2	2	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
3	3	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4	4	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooo
5	5	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooo
6	6	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	*****oo*****oooo
7	7	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8	8	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9	9	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10	10	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11	11	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oo*****oooooooo
12	12	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	<input 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"/>	<input 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"/>	<input type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, % conv

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

6.5. Alignment page

6.5.1. 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 shows the QUMA Alignment result page with four main sections:

- A) Summary information:** Displays basic alignment statistics: Bisulfite sequence name (SeqBisulfite_seq_01), Bisulfite sequence (containing CpG conversion and detection of removal of the genomic sequence), Length of bisulfite sequence (98), Length of target genome sequence (423), Aligned region of bisulfite sequence (41 - 409), Alignment direction (Forward), SeqCpG (319), Unconverted CpG (32), and % identity (99.97 / 99.97%). It also shows the sequence logo.
- B) Target genome sequence:** Shows the genomic DNA sequence with CpG sites highlighted in blue.
- C) Bisulfite sequence:** Shows the bisulfite-treated DNA sequence where methylated Cs are shown in blue, unmethylated Cs in gray, and unconverted Cs (A, C, T) in black.
- D) Alignment:** A detailed view of the aligned region showing the target genome sequence (Genome) and the bisulfite sequence (Bisulfite). The alignment highlights the presence of methylated CpG sites.

6.5.2. Download alignment data

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

The screenshot shows the QUMA Alignment result page. At the top, there is a summary table with the following data:

Bisulfite sequence name	Omb_16abbb_seq_05	group2	
C => T conversion (conversion and detection of forward strand of the genomic sequence)			
Length of bisulfite sequence	983	Length of target genome sequence	453
Aligned region of bisulfite sequence	45 - 498	Alignment direction	forward
Me-CpG	5/19	Unconverted CpH	2/127
mismatch (gap) / alignment length (% identity)	10 (7) / 457 (97.8 %)		
Methylation pattern	oooooooooooo		

Below the table, there is a section titled "Target genome sequence" containing the DNA sequence:

```
CAAGAGACCTGAGCTTGGAGAAACTGGATCTTCAGTTCTAGGTAGTCGAGGGGGAAAG  
GCATCACACCATAAATCGCATTGACACCGCAGCTTGAAAGCTGGGTTTTCTAGCGA  
GCTCAGAGGCTCTGGTAGATTCTATCCTTAGTCTCGCTCTTCGCCCTTCCCCAACAA  
GACACAGGTTTCCCTCGAAAAAAACACACCCGGAAAGCTGTCACTCAATCCCCAACAA  
GCGTGGTGGCTTTGCAATCTGGGAGTCGCCAACATCACACATATGGCACATTCTAGCC  
CTCCAATCTAGGGTTGTGAAATGCTCCCAACCGATCCGATCCCTAAGAACAGAA  
GACCTCTAGACAATCGAAACTCGAGCATCAAAGCATCACAGCACATACAATCACAA  
TTATGTGTCCTAGCTGTCATCCCCACT
```

To the right of the browser window, a detailed "Alignment results No. 21" panel is shown, listing various parameters and the target genome sequence in its entirety.

6.5.3. Alignment data

Downloaded alignment data file can be opened byTextEdit (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. [Binary 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 withTextEdit (Macintosh), WordPad (Windows) or many word processors. Plain text file can be created withTextEdit (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.

```
CAGTCGGCAGGCCGGGTTAACGCGCCAAGTAAACGTAGCGCAGCGA  
TCGGCGCCGGAGATTCGCGAACCGACACTCCGCGCCGCCGCCAG  
GACCCGCGCGCGATCGCGGCCGCGCTACAGCCAGCCTCACTGGCGC  
CGGGCGAGCGCACGGCGCTC
```

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  
ACTCCCCGTGCGCGCCGGCCCGTAGCGTCCCTCGTCGCCGCCCTCGTCT  
CGCAGCCGCAGCCCGGTGGACGCTCTCGCCTGAGCGCCGCGACTAGCC  
CGGGTGGCCCAGTGGCGCGGGCGAGCGCACGGCGCTCCAGTCCGGCA  
GCGCCGGGTTAACGCGCCAAGTAAACGTAGCGCAGCGATCGCGCCGG  
AGATTCGCGAACCGACACTCCGCGCCGCCAGGACCCGCG  
GCGATCGCGCGCCCGCTACAGCCAGCCTCACGACAGGCCGCTGAGGC  
TTGTGCCAGACCTTGAAACCTCAGGTATATACTTCCAGACGGCGGGAT  
CTCCCCCTCCCCATCCATAGTGCCTTGGGACCAAATCCAGGGCCTCTTT  
CAGGAAACAATGAAGGGAGACAGCAGACATCTGAATGAAGAAGAGGGTGC  
CAGCGGGTATGAGGAGTCATTATCGTTAATGGGAACTTCAGTGACCAGT  
CCTCAGACACGAAGGATGCTCCCTCACCCCCAGTCTGGAGGCAATCTGC  
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
    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 gcccggccac ccggccggcc acacggcaga gccgcctgaa
    61 gcccagcgct gaggtgcac tttccgagg gcttgacatc agggtctatg tttaagtctt
    121 agctcttgct tacaaagacc acggcaattc cttctctgaa gccctcgtag ccccacagcg
    181 ccctcgcagc cccagcctgc
//
```

8.4. Multi-FASTA format

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

ex.

```
>sequence1
ACTCCCCGTGCGCGCCGGCCCGTAGCGTCCTCGTGCAGCCCTCGTCTCGCAGCCGCA
GCCCGCGTGGACGCTCTGCCTGAGGCCGCGGACTAGCCGGGTGGCC
>sequence2
CAGTCCGGCAGGCCGGGTTAACGCCCCAAGTAAACGTAGCGCAGCGATGGCGCCGG
AGATTCGCGAACCCGACACTCCCGCCGCCGCCAGGACCCGCGCGATCGCGG
CGCCGCGCTACAGCCAGCCTCACTGGCGCGGGCGAGCGCACGGCGCTC
>sequence3
CACGACAGGCCGCTGAGGCTTGTGCCAGACCTGGAAACCTCAGGTATATACTTCCA
GACGCGGGATCTCCCCTCCCC
>sequence4
CAGCAGACATCTGAATGAAGAAGAGGGTGCCAGCGGGTATGAGGAGTGCATTATCGTTAA
TGGGAACCTCAGTGACCAGTCCTCAGACACGAAGGATGCTCCCTACCCCCAGTCTTGA
GGCAATCTGCACAGAGCCAGTCTGCACACC
```

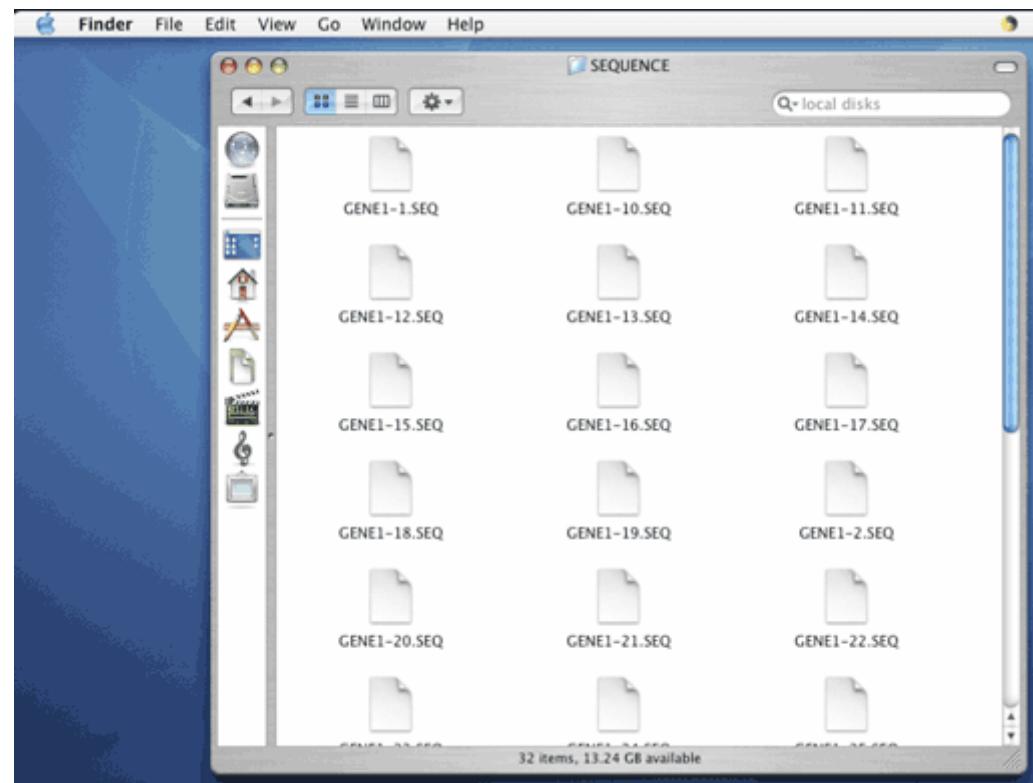
8.5. Zipped archive of sequence files

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".

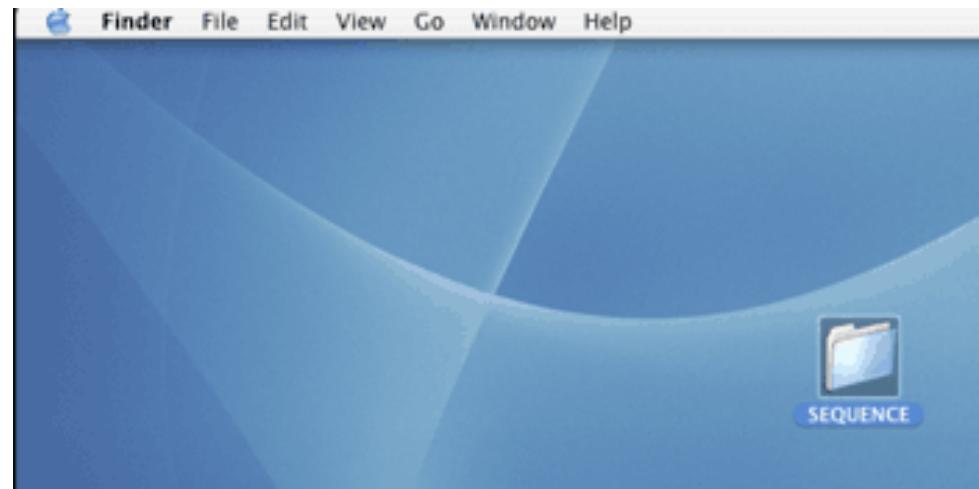
8.6. How to create zipped archive (Macintosh)

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".)



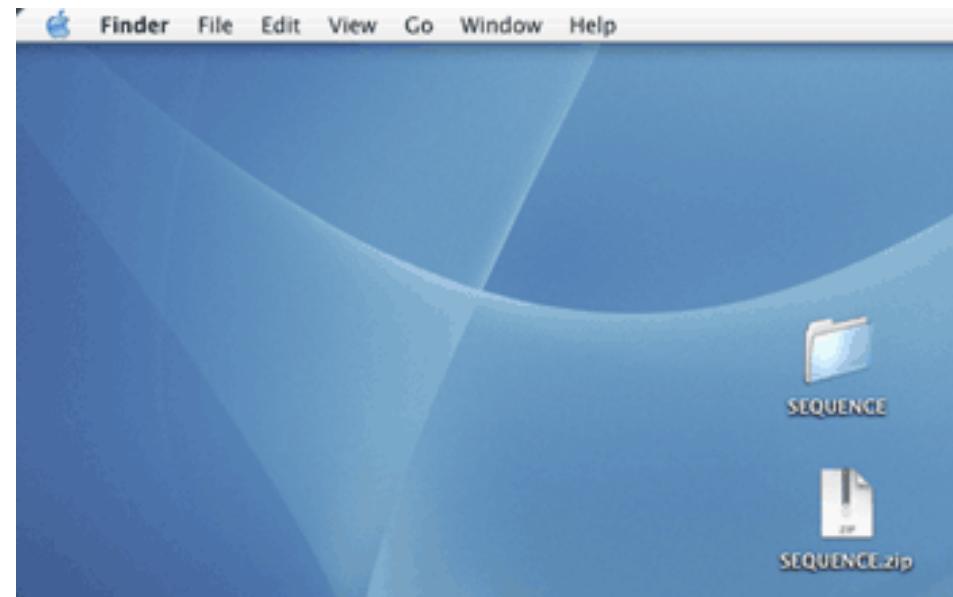
2. Click to select the folder.



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.



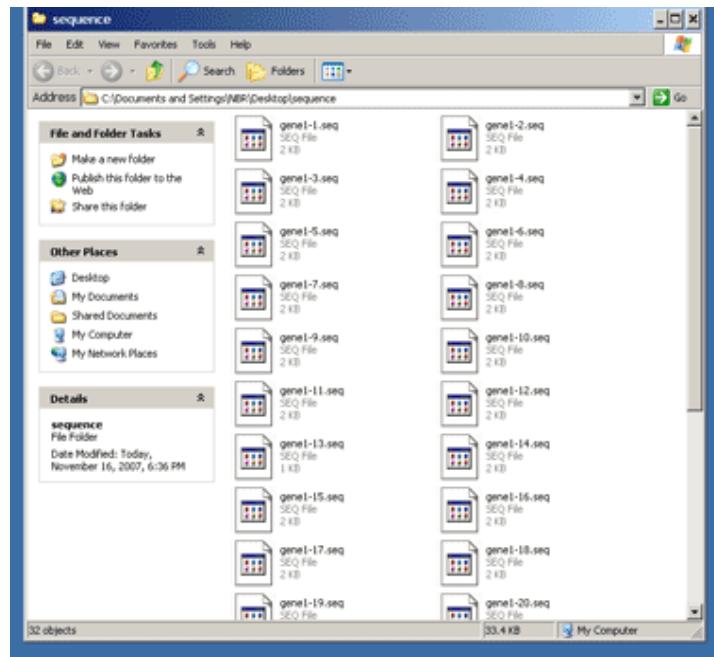
8.6.2. Other Mac OS

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

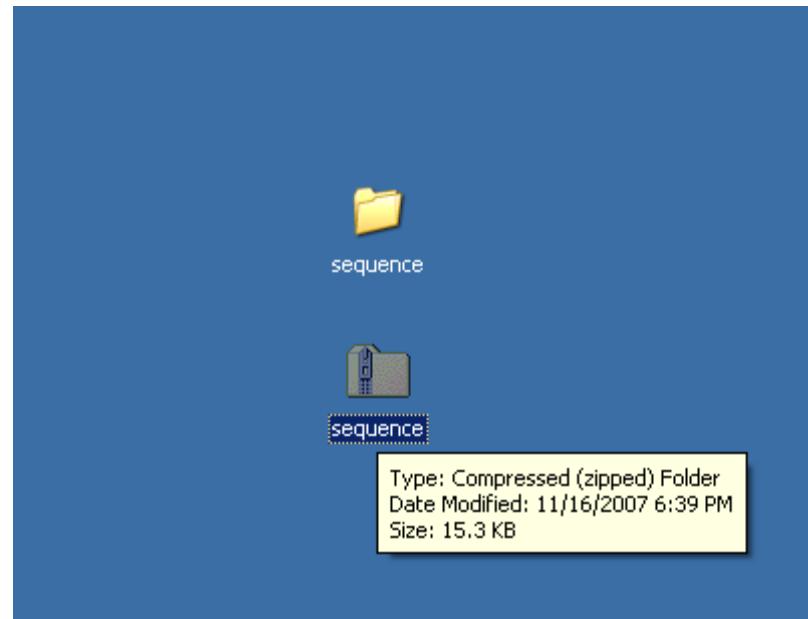
1. Put bisulfite sequence files of [8.2. FASTA](#) or [8.1. Plain sequence](#) format into a folder. (Acceptable file extension of sequence file is ".seq", ".fa", ".fas", ".fasta" or ".txt".)



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 (exampled 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:

$$p = \frac{a+bC_a * c+dC_c}{a+b+c+dC_{a+c}} = \frac{13C_{12} 10C_4}{23C_{16}} = \frac{(13! 10! 16! 7!)}{(12! 1! 4! 6! 23!)} = 0.0111357212$$

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
group1	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)
group2	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): R₁ and R₂.

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	3	3	212.5 (=R ₁)
	group2	2	2	2	0	3	1	0	0	0	0	0	63.5 (=R ₂)
	total	2	2	2	2	3	2	1	1	1	3	3	

Third, determine temporary U-value, U₁ and U₂, 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 U₁ and U₂ 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 Whitney 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 ₁)
	group2	1	2	0	2	0	0	0	0	5	19 (=R ₂)
	total	1	2	2	2	1	1	1	1	11	

$$U_1 = n_1 * n_2 + n_1 * (n_1 + 1) / 2 - R_1 = 4$$

$$U_2 = n_1 * n_2 + n_2 * (n_2 + 1) / 2 - R_2 = 26$$

$$U = \min(U_1, U_2) = 4$$

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	2/0	0/2	1/0	1/0	1/0	1/0	47/19	4	0.00216
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

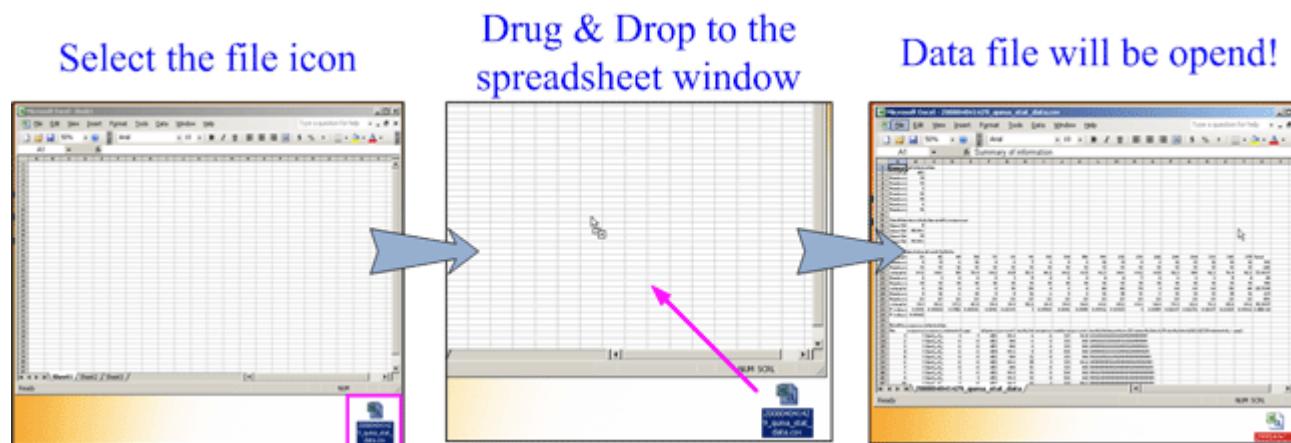
In many case, CSV formatted analysis data file can be opened from Microsoft Excel by double-clicking the file icon. If not, try the "drug & drop" procedure indicated below.

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").