

QUantification tool for Methylation Analysis

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

QUMA User's manual

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Contact information

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Contents

1. About QUMA	5
2. Quick start.....	6
2.1. Select a genomic sequence file	6
2.2. Select a bisulfite sequence file	6
2.3. Submit.....	7
3. Supported browsers	7
4. Overview	8
5. Methylation status analysis mode.....	9
5.1. Main features.....	9
5.2. Top page	10
5.3. Top page simple.....	11
5.3.1. Genomic sequence file 1	11
5.3.2. Genomic sequence file 2	11
5.3.3. Bisulfite sequences file 1	12
5.3.4. Bisulfite sequences file 2	12
5.3.5. Submit	13
5.4. Top page option	13
5.4.1. Show options.....	13
5.4.2. Optional fields	14
5.4.3. Hide options	15
5.4.4. Genomic sequence	15
5.4.5. Genomic sequence file 1	16
5.4.6. Genomic sequence file 2	16
5.4.7. Bisulfite sequences	17
5.4.8. Bisulfite sequences file 1	17
5.4.9. Bisulfite sequences file 2	18
5.4.10. Conditions to exclude bisulfite sequences.....	18
5.4.11. Strand of bisulfite conversion	19
5.5. Analysis result page	20
5.5.1. Overview of analysis result page 1	20
5.5.2. Overview of analysis result page 2	21
5.5.3. Change methylation status figure 1	21
5.5.4. Change methylation status figure 2	22
5.5.5. Download methylation status figure	23
5.5.6. Overview of analysis result page 3	23
5.5.7. Show alignment.....	25
5.5.8. Include/exclude bisulfite sequence 1	25
5.5.9. Include/exclude bisulfite sequence 2	26
5.5.10. Change the order of bisulfite sequences 1	26
5.5.11. Change the order of bisulfite sequences 2	27
5.5.12. Download alignments data.....	27
5.5.13. Alignments data	28
5.5.14. Download analysis data	29
5.5.15. Analysis data	29
5.5.16. Download methylation pattern figure	30

5.5.17. Methylation pattern figure	30	5.8.10. Option of figure 3	41
5.5.18. Go to figure page	31	5.8.11. Figure 4	42
5.6. Result page options	31	5.8.12. Option of figure 4	42
5.6.1. Show options 1	31	6. Statistical analysis mode	43
5.6.2. Show options 2	32	6.1. Main features	43
5.6.3. Hide options	32	6.2. Top page	44
5.6.4. Change the order of bisulfite sequences 1	33	6.2.1. Show options	44
5.6.5. Change the order of bisulfite sequences 2	33	6.2.2. Optional fields	44
5.6.6. Conditions to exclude bisulfite sequences 1	34	6.2.3. Genomic sequence	45
5.6.7. Conditions to exclude bisulfite sequences 2	34	6.2.4. Genomic sequence file 1	45
5.7. Alignment page	35	6.2.5. Genomic sequence file 2	46
5.7.1. Overview of alignment page	35	6.2.6. First bisulfite sequence group	46
5.7.2. Download alignment data	36	6.2.7. File of first bisulfite sequence group 1	47
5.7.3. Alignment data	36	6.2.8. File of first bisulfite sequence group 2	47
5.8. Figure page	37	6.2.9. Second bisulfite sequence group	48
5.8.1. Download methylation pattern figure	37	6.2.10. File of second bisulfite sequence group 1	48
5.8.2. Change methylation pattern figure 1	37	6.2.11. File of second bisulfite sequence group 2	49
5.8.3. Change methylation pattern figure 2	38	6.2.12. Conditions to exclude bisulfite sequences	49
5.8.4. Show options	38	6.2.13. Strand of bisulfite conversion	50
5.8.5. Figure 1	39	6.2.14. Submit	50
5.8.6. Option of figure 1	39	6.3. Statistical analysis result page	51
5.8.7. Figure 2	40	6.3.1. Overview of statistical analysis result page 1	51
5.8.8. Option of figure 2	40	6.3.2. Overview of statistical analysis result page 2	52
5.8.9. Figure 3	41	6.3.3. Change methylation status figure 1	53

6.3.4. Change methylation status figure 2	53	7.1. Genomic sequence	67
6.3.5. Download comparative methylation status figure ..	54	7.2. Bisulfite sequences	68
6.3.6. Overview of statistical analysis result page 3	55	8. Sequence format	69
6.3.7. Show alignment	57	8.1. Plain sequence format	69
6.3.8. Include/exclude bisulfite sequence 1	57	8.2. FASTA format	69
6.3.9. Include/exclude bisulfite sequence 2	58	8.3. GenBank format	70
6.3.10. Change the order of bisulfite sequences 1	58	8.4. Multi-FASTA format	71
6.3.11. Change the order of bisulfite sequences 2	59	8.5. Zipped archive of sequence files	72
6.3.12. Download alignments data	59	8.6. How to create zipped archive (Macintosh)	72
6.3.13. Alignments data	60	8.6.1. Mac OS X 10.3 and later	72
6.3.14. Download statistical analysis data	61	8.6.2. Other Mac OS	74
6.3.15. Statistical analysis data	61	8.7. How to create zipped archive (Windows)	75
6.4. Statistical analysis result page options	62	8.7.1. Windows Me/XP/Vista	75
6.4.1. Show options 1	62	8.7.2. Other Windows	76
6.4.2. Show options 2	62	9. Statistical test	77
6.4.3. Hide options	62	9.1. Fisher's exact test	77
6.4.4. Change the order of bisulfite sequences 1	63	9.2. Mann-Whitney U-test	79
6.4.5. Change the order of bisulfite sequences 2	63	10. Other	84
6.4.6. Conditions to exclude bisulfite sequences 1	64	10.1. How to open a CSV file	84
6.4.7. Conditions to exclude bisulfite sequences 2	64	10.1.1. Mac OS	84
6.5. Alignment page	65	10.1.2. Windows	84
6.5.1. Overview of alignment page	65		
6.5.2. Download alignment data	66		
6.5.3. Alignment data	66		
7. Input data	67		

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

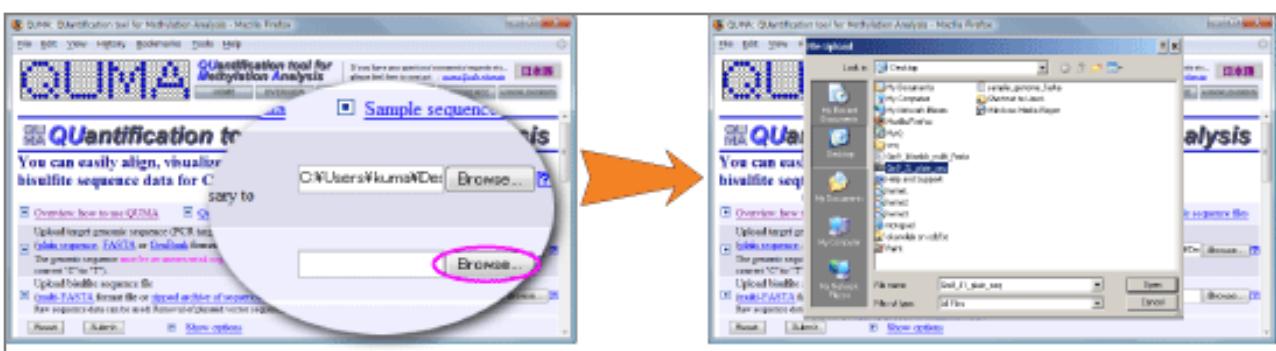
2.1. Select a genomic sequence file

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.

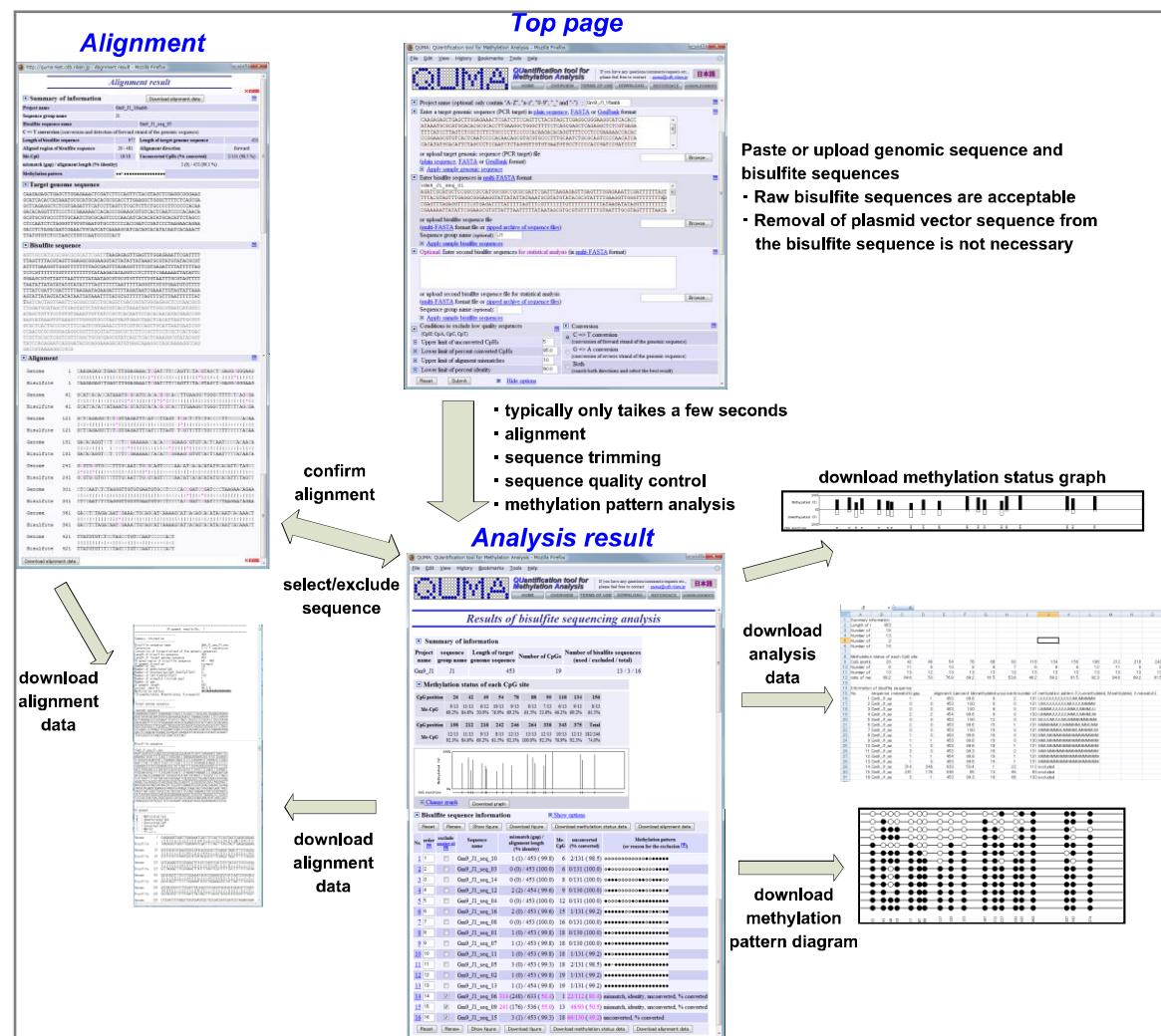
The screenshot displays three main sections of the QUMA web application:

- Top page:** Shows the main navigation bar and a summary of information for a project named "GrpJ1_16sub". It includes tables for "Statistical data" and "P-value of Fisher's exact test".
- Methylation status analysis:** This section is titled "Results of bisulfite sequencing analysis". It shows a table of CpG positions and their methylation status, along with a bar chart of methylation percentages. A "Methylation status" button is highlighted in blue.
- Statistical analysis:** This section is titled "Results of statistical analysis". It shows a table of CpG positions and their methylation status, along with a bar chart of methylation percentages. A "Statistical analysis" button is highlighted in blue.

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 application running in Mozilla Firefox. The title bar reads "QUMA: QUantification tool for Methylation Analysis - Mozilla Firefox". The menu bar includes File, Edit, View, History, Bookmarks, Tools, and Help. A logo consisting of a grid of blue dots forming the letters "QUMA" is displayed. To its right, the text "QUantification tool for Methylation Analysis" is written in blue. Below the logo are several navigation buttons: HOME, OVERVIEW, TERMS OF USE, DOWNLOAD, REFERENCE, and ACKNOWLEDGEMENTS. A link to "日本語" (Japanese) is also present. A message in the top right corner says, "If you have any questions/comments/requests etc., please feel free to contact : quma@cdb.riken.jp". The main content area features a large blue header with the text "QUantification tool for Methylation Analysis". Below it, a sub-header reads "You can easily align, visualize and quantify bisulfite sequence data for CpG methylation analysis". There are four checkboxes at the top of the input section: "Overview: how to use QUMA", "Quick start", "Execute with sample sequence data", and "Sample sequence files". The first checkbox is checked. Below these are two input fields for target genomic sequence: one for plain sequence (in FASTA or GenBank format) and another for multi-FASTA or zipped archive of sequence files. Both fields have "Browse..." buttons and help icons. A note specifies that the genomic sequence must be an unconverted sequence between PCR primer pair. The bottom of the input section has "Reset", "Submit", and "Show options" buttons.

5.3. Top page simple

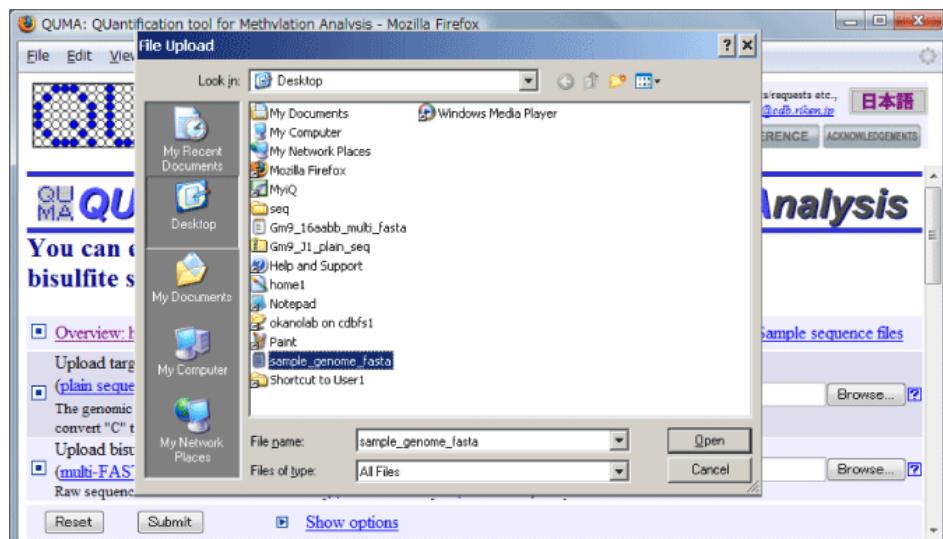
5.3.1. Genomic sequence file 1

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](#)".



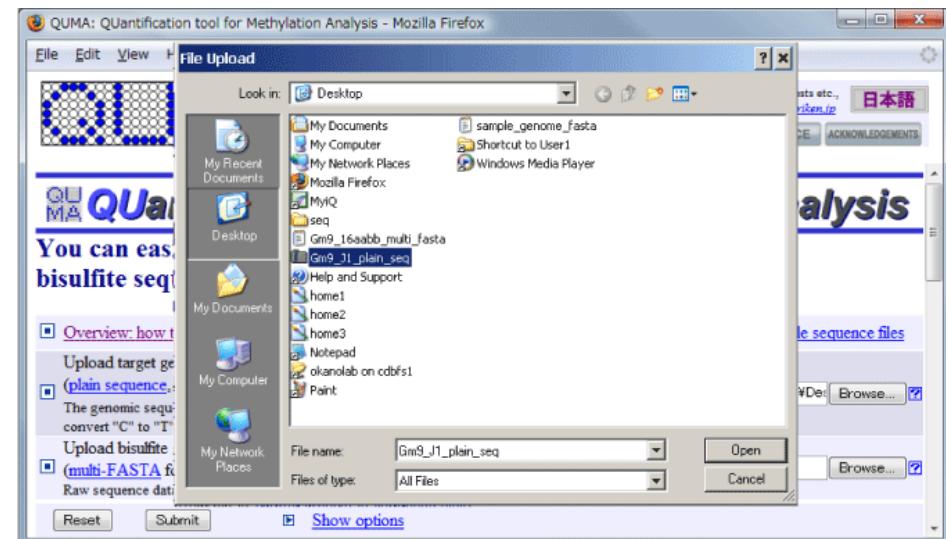
5.3.3. Bisulfite sequences file 1

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.

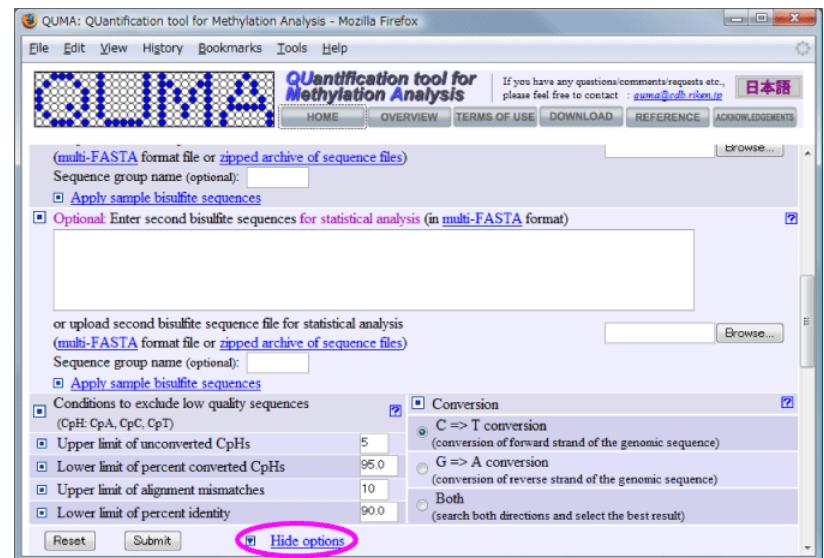
The third text input field is used only for the [Statistical analysis mode](#).

The screenshot shows the QUMA web interface with several optional input fields highlighted:

- Project name (optional):** A text input field with placeholder text "only contain 'A-Z', 'a-z', '0-9', '_' and '-'".
- Enter a target genomic sequence (PCR target):** A text input field with placeholder text "in plain sequence, FASTA or GenBank format". Below it are two options:
 - [Plain sequence](#)
 - [FASTA](#)
 - [GenBank](#)
- Enter bisulfite sequences in multi-FASTA format:** A text input field with placeholder text "or upload bisulfite sequence file (multi-FASTA format file or zipped archive of sequence files)". Below it are two options:
 - [Apply sample bisulfite sequences](#)
 - [Optional: Enter second bisulfite sequences for statistical analysis \(in multi-FASTA format\)](#)
- Conditions to exclude low quality sequences:** A section with five checkboxes and dropdown menus:
 - CpG: CpA, CpC, CpT (Upper limit of unconverted CpGs: 5%)
 - Upper limit of percent converted CpGs (95.0%)
 - Lower limit of percent converted CpGs (10%)
 - Upper limit of alignment mismatches (90.0%)
 - Lower limit of percent identity (90.0%)
- Conversion:** A section with three radio button options:
 - 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)

5.4.3. Hide options

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

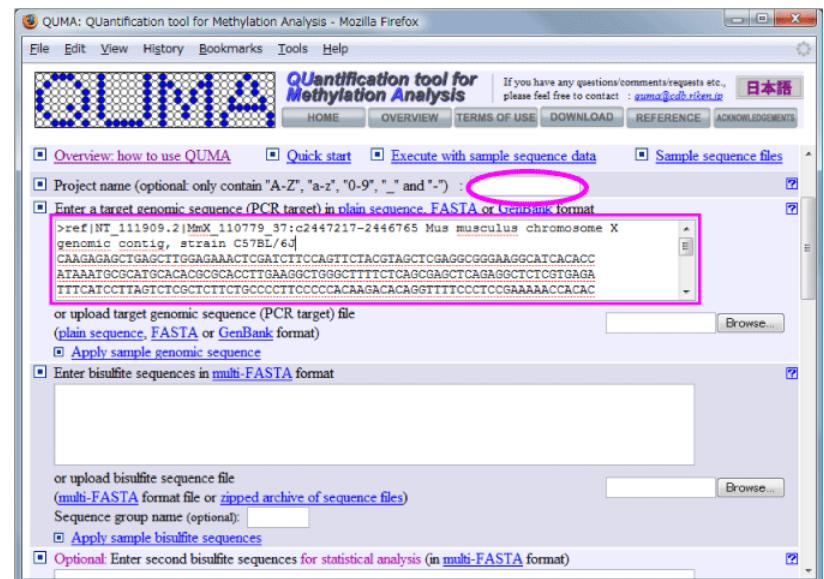


5.4.4. Genomic sequence

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](#)".



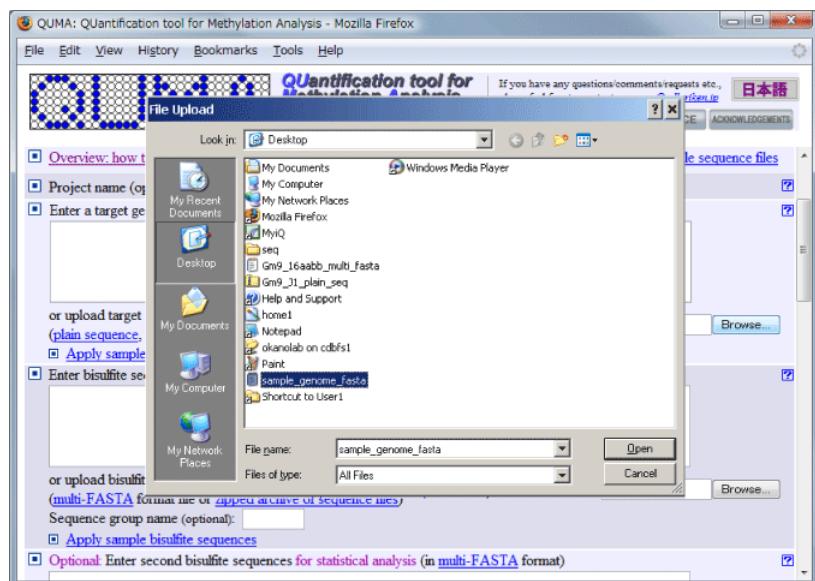
5.4.5. Genomic sequence file 1

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](#)".

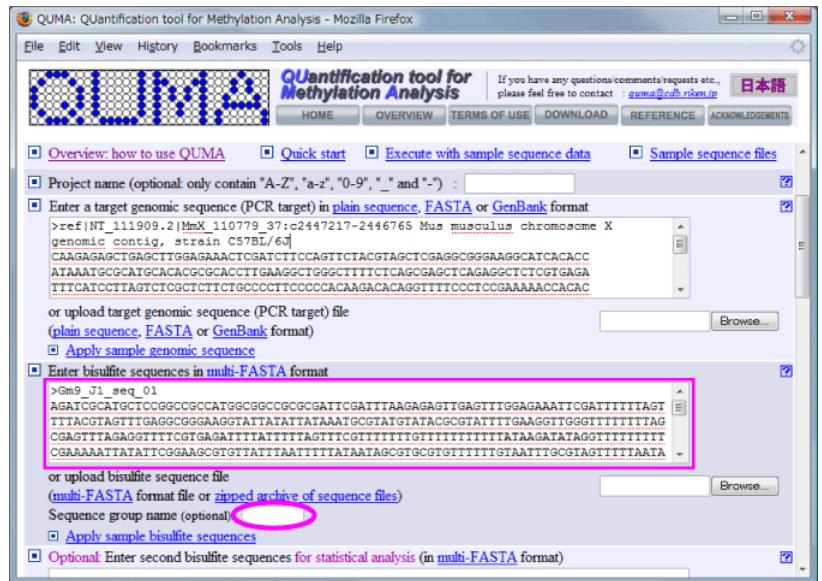


5.4.7. Bisulfite sequences

Input a group name of bisulfite sequences (optional).

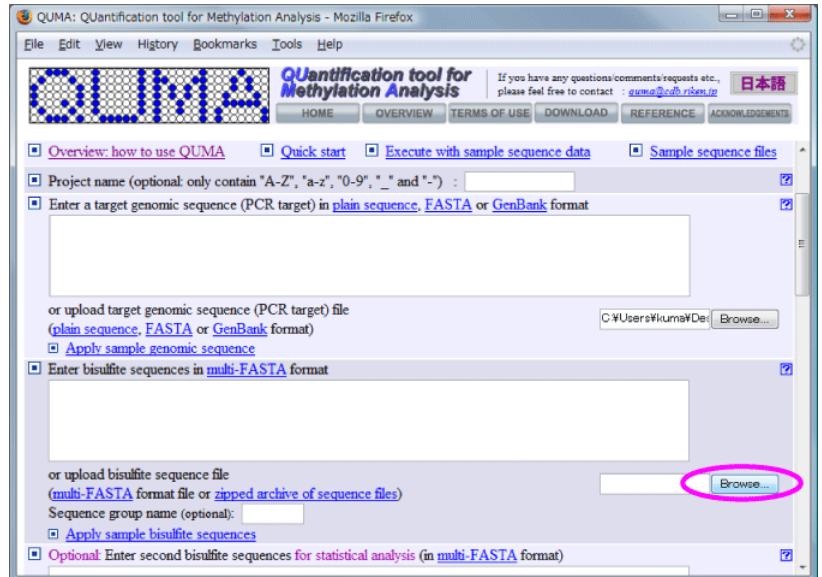
The bisulfite sequences can be input by two ways of 1) direct input and 2) upload.

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



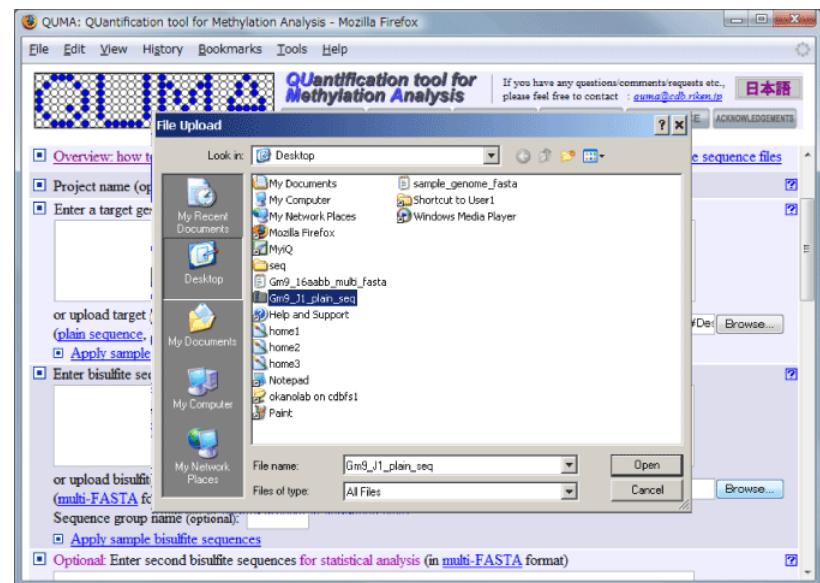
5.4.8. Bisulfite sequences file 1

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



5.4.9. Bisulfite sequences file 2

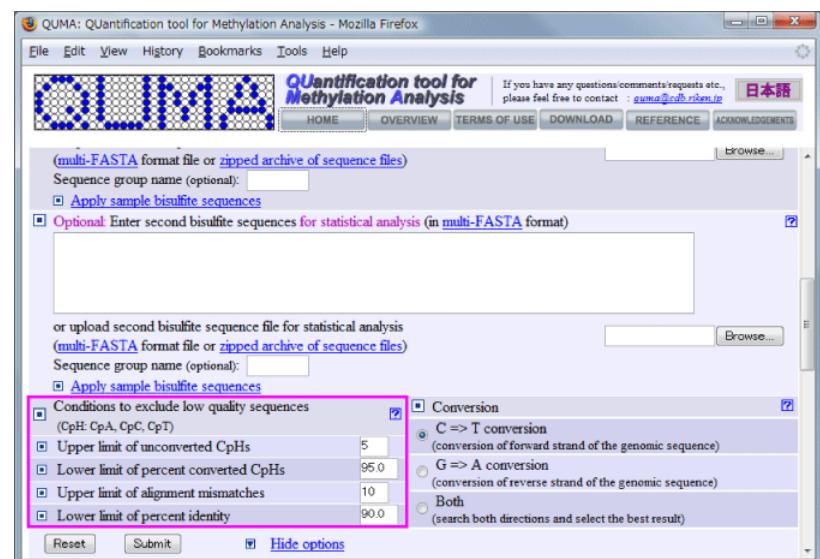
Select a file of bisulfite sequences. Acceptable file formats are [8.4. Multi-FASTA](#) or [8.5. Zipped archive of sequence files](#). See also "[7.2. Bisulfite sequences](#)", "[8.6. How to create zipped archive \(Macintosh\)](#)" and "[8.7. How to create zipped archive \(Windows\)](#)".



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



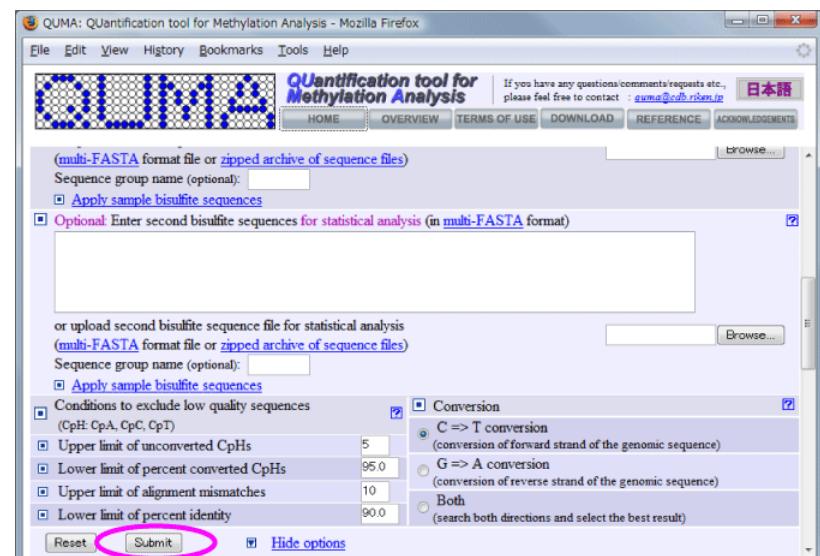
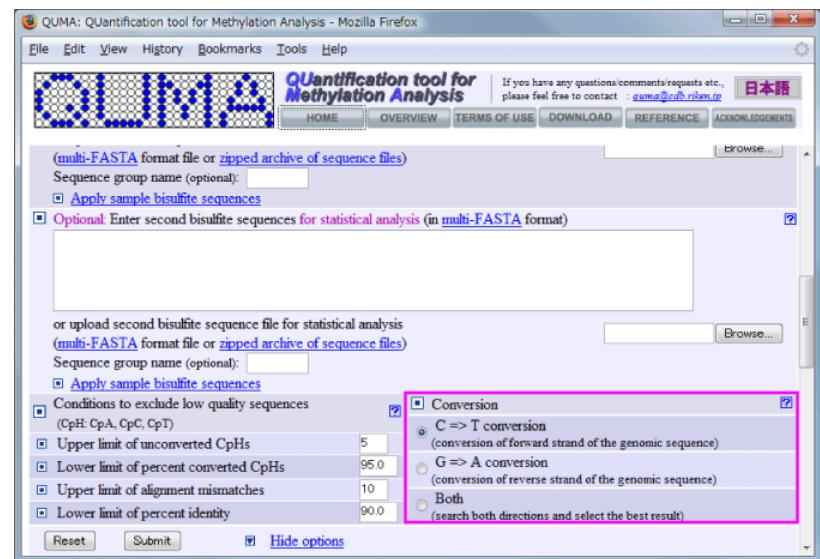
5.4.11. Strand of bisulfite conversion

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



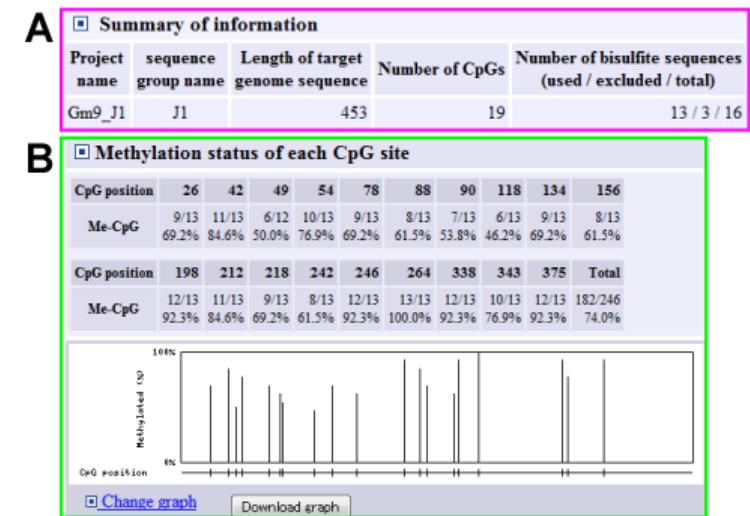
5.5.2. Overview of analysis result page 2

A) Summary of information

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

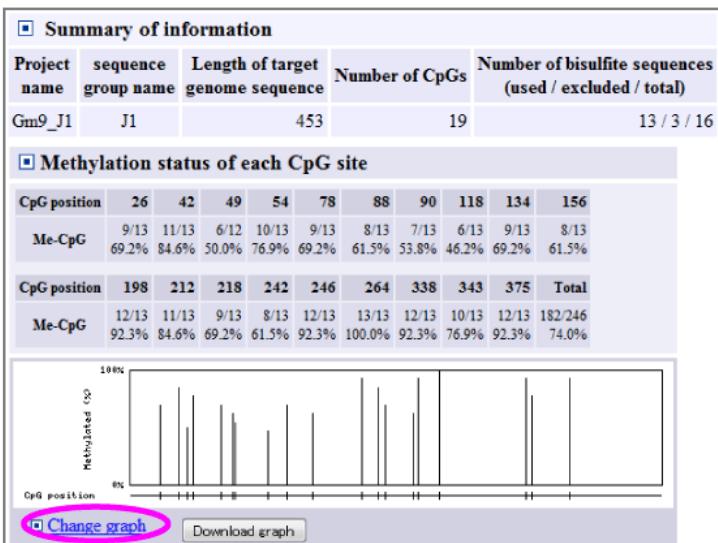
B) Methylation status of each CpG sites

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



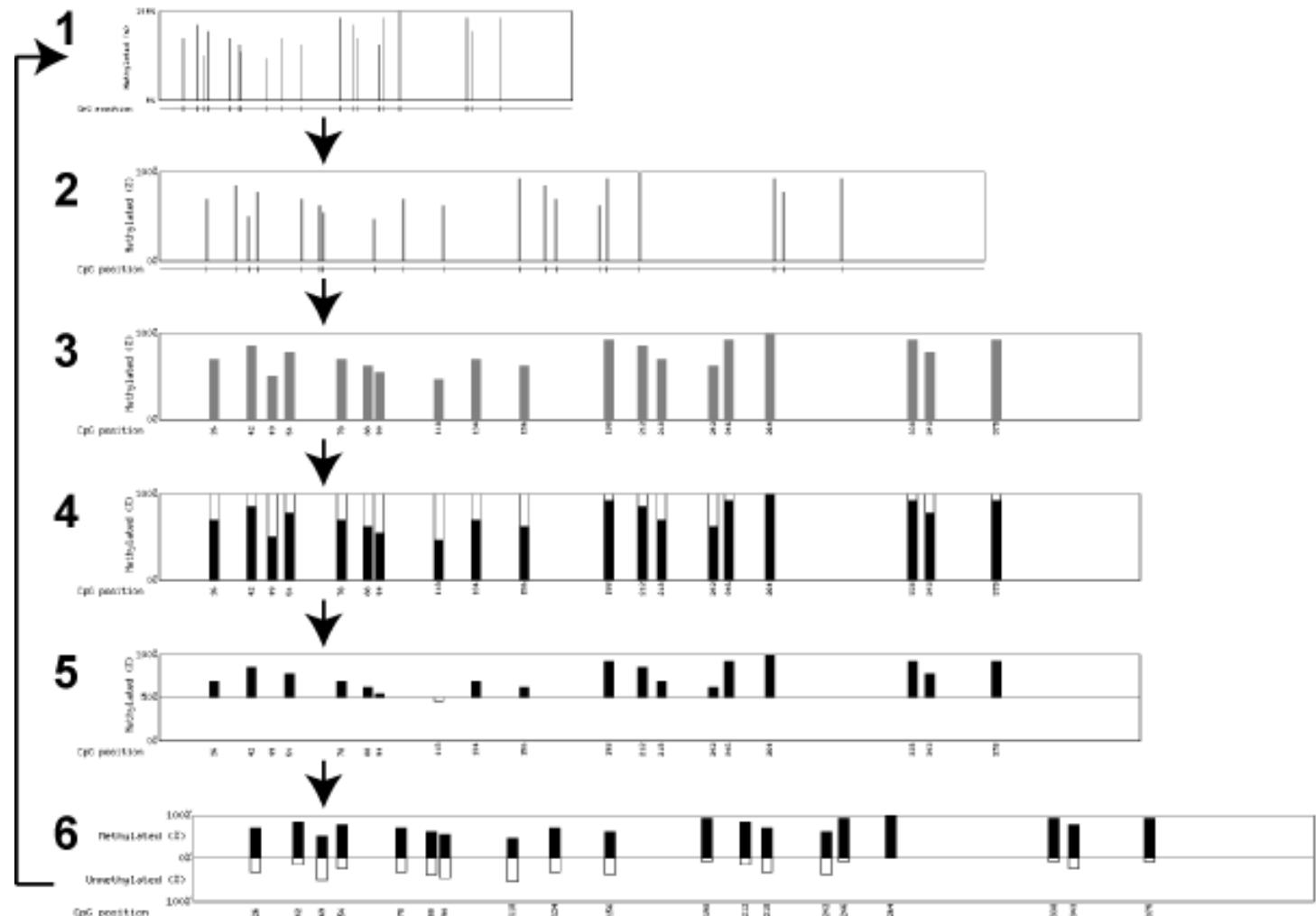
5.5.3. Change methylation status figure 1

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



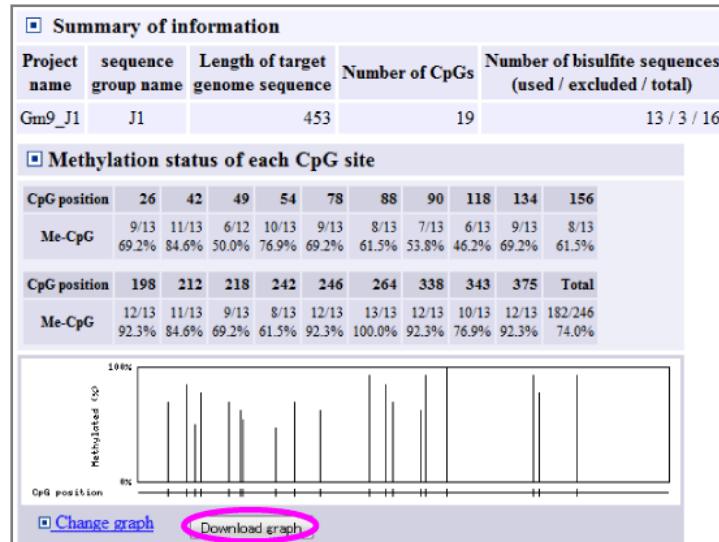
5.5.4. Change methylation status figure 2

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"/> unselect all <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)	oooooooooooooo*****
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooo*****
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooo*****
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooo*****
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	*****oooooo*****
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	*****oooooo*****
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	*****oooooo*****
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	*****oooooo*****
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	*****oooooo*****
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	***x*****oooooo*****
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	*****oooooo*****
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	*****oooooo*****
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)	ooo-xoooooooooooo
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)	ooo-xoooooooooooo
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							Show options
	Reset	Renew	Show figure	Download figure	Download methylation status data	Download alignment data	
No.	order	exclude methylated	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)	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_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooo
13	13	<input checked="" type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	user desired
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.1)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (50.5)	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							Show options
	Reset	Review	Show figure	Download figure	Download methylation status data	Download alignment data	
No.	order	exclude mismatch all	Sequence name	mismatch (gap)/ alignment length (% identity)	Me- CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion)
1	6	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooo•oooooooo
2	5	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooo•oooooooo
3	4	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooo•oooooooo
4	3	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooo•oooooooo
5	2	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooo•oooooooo
6	1	<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)	oooooooooooooo•oooooooo
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

5.5.11. 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
Reset	Renew	Show figure	Download figure	Download methylation status data	Download alignment data		
No.	order	exclude mismatch all <input type="checkbox"/>	Sequence name	mismatch (gap) / alignment length (% identity)	Me- CpG	unconverted (% converted)	Methylation pattern (or reason for the exclusion ?)
1		<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
2		<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
3		<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
4		<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
5		<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooo
6		<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooooo
7		<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
8		<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
9		<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
10		<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
11		<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oo-----oooo
12		<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13		<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14		<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15		<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16		<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

5.5.15. Analysis data

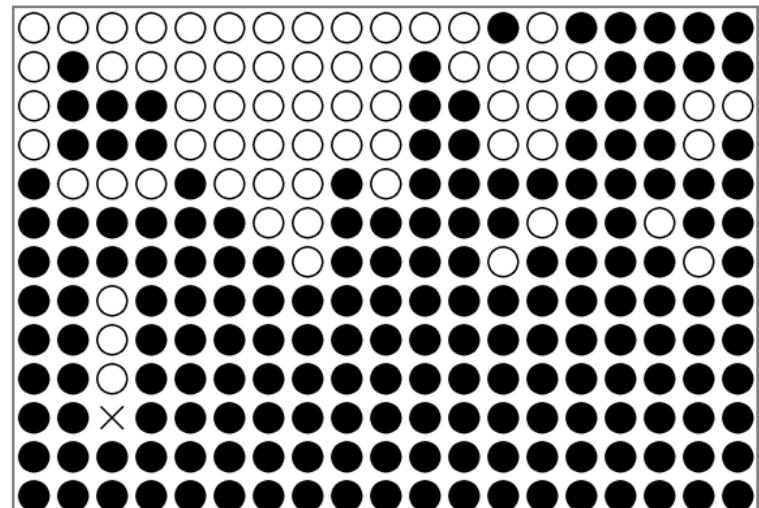
Downloaded analysis data file can be opened by Microsoft Excel, [OpenOffice/StartSuite](#) or other spreadsheet software (**CSV** file format).

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						
No.	order	exclude unselected	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) ○○○○●○○○○●○○○○
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) ○○○○●○○○○●○○○○
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0) ○○○○●○○○○●○○○○
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2) ○○○○●○○○○●○○○○
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5) ○○=○○○○●○○○○
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2) ○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



5.5.17. 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)	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)	ooo•oooooooooooo
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

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)	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)	ooo•oooooooooooo
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

5.6.2. Show options 2

Optional fields will appear.

Bisulfite sequence information							
<input checked="" type="checkbox"/> 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 <input type="radio"/> Upper limit of unconverted CpHs : 5 <input type="radio"/> number of unconverted CpHs <input type="radio"/> percent converted CpHs <input type="radio"/> Lower limit of percent converted CpHs : 95.0 <input type="radio"/> number of mismatches <input type="radio"/> percent identity <input type="radio"/> Upper limit of alignment mismatches : 10 <input type="radio"/> sequence name <input type="radio"/> <input type="radio"/> Lower limit of percent identity : 90.0							
<input checked="" type="radio"/> ascending order <input type="radio"/> descending order							
<input type="button"/> Reset <input type="button"/> Renew <input type="button"/> Show figure <input type="button"/> Download figure <input type="button"/> Download methylation status data <input type="button"/> Download alignment data							
No.	order	exclude <input checked="" type="checkbox"/>	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	0/131 (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*oooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

5.6.3. Hide options

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

Bisulfite sequence information							
<input 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"/> Upper limit of unconverted CpHs : 5 <input type="radio"/> number of unconverted CpHs <input type="radio"/> percent converted CpHs <input type="radio"/> Lower limit of percent converted CpHs : 95.0 <input type="radio"/> number of mismatches <input type="radio"/> percent identity <input type="radio"/> Upper limit of alignment mismatches : 10 <input type="radio"/> sequence name <input type="radio"/> <input type="radio"/> Lower limit of percent identity : 90.0							
<input checked="" type="radio"/> ascending order <input type="radio"/> descending order							
<input type="button"/> Reset <input type="button"/> Renew <input type="button"/> Show figure <input type="button"/> Download figure <input type="button"/> Download methylation status data <input type="button"/> Download alignment data							
No.	order	exclude <input checked="" type="checkbox"/>	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*oooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
13	13	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconverted, % converted

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

Bisulfite sequence information

Sorting conditions: CpH: CpA, CpC, CpT

Conditions to exclude low quality sequences

No.	order	exclude number 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)	ooooooooooooo•ooooooooooooo
2	2		Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	ooooooooooooo•ooooooooooooo
3	8		Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	ooooooooooooo•ooooooooooooo
4	4		Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	ooooooooooooo•ooooooooooooo
5	5		Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	ooooooooooooo•ooooooooooooo
6	6		Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	ooooooooooooo•ooooooooooooo
7	7		Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	ooooooooooooo•ooooooooooooo
8	8		Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooo•ooooooooooooo
9	9		Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooo•ooooooooooooo
10	10		Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	ooooooooooooo•ooooooooooooo
11	11		Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	ooooooooooooo•ooooooooooooo
12	12		Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	ooooooooooooo•ooooooooooooo
13	13		Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	ooooooooooooo•ooooooooooooo
14	14	✓	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, identity, unconverted, % converted
15	15	✓	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, identity, unconverted, % converted
16	16	✓	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

Bisulfite sequence information

Sorting conditions: CpH: CpA, CpC, CpT

Conditions to exclude low quality sequences

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

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

5.6.5. Change the order of bisulfite sequences 2

The change is reflected.

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 parameter					
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)	oooooooooooooo.....ooooo
4	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooo.....ooooo
5	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooo.....ooooo
6	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooo.....ooooo
7	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooo.....ooooo
8	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo.....ooooo
9	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo.....ooooo
10	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooo.....ooooo
11	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	ooo.....oooooooooo.....ooooo
12	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooo.....ooooo
13	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooo.....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 parameter					
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)	oooooooooooooo.....ooooo
2	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooo.....ooooo
3	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooo.....ooooo
4	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooo.....ooooo
5	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo.....ooooo
6	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooo.....ooooo
7	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooo.....ooooo
8	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooo.....ooooo
9	<input checked="" type="radio"/>	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooo.....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

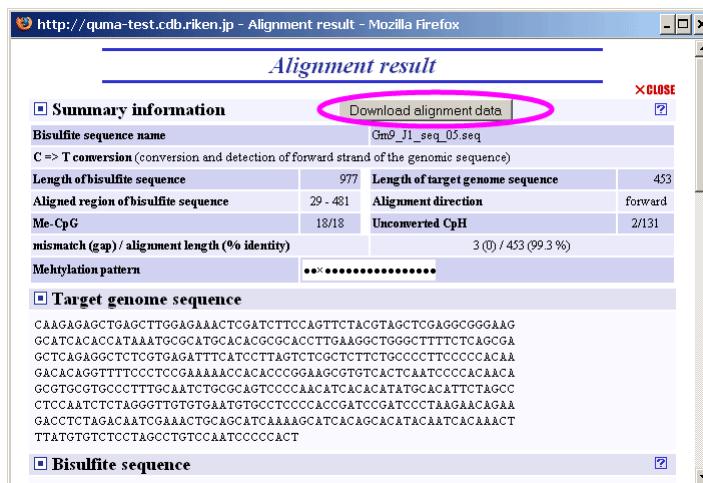
Methylated C of CpG site, unmethylated C of CpG site, Unconverted C (CpA, CpC, CpT) are indicated as different colors.

The screenshot shows the 'Alignment result' page from the QUMA web interface. It includes four main sections:

- A Summary information:** Displays basic statistics: Bisulfite sequence name (Self_11_35_57.mq), Length of Bisulfite sequence (871), Aligned region of Bisulfite sequence (29-481), Length of target genome sequence (481), M-CpG (107X), mismatch (gap) / alignment length (% identity) (3 (0) / 65 (92 %)), and Modification pattern (Methylation).
- B Target genome sequence:** Shows the genomic DNA sequence with CpG sites highlighted in green.
- C Bisulfite sequence:** Shows the bisulfite-treated DNA sequence where methylated Cs are shown in red, unmethylated Cs in blue, and unconverted Cs (A, C, T) in black.
- D Alignment:** A detailed view of the aligned regions, showing the sequence for each strand (Genome and Bisulfite) with color-coded base pairs.

5.7.2. Download alignment data

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



5.7.3. Alignment data

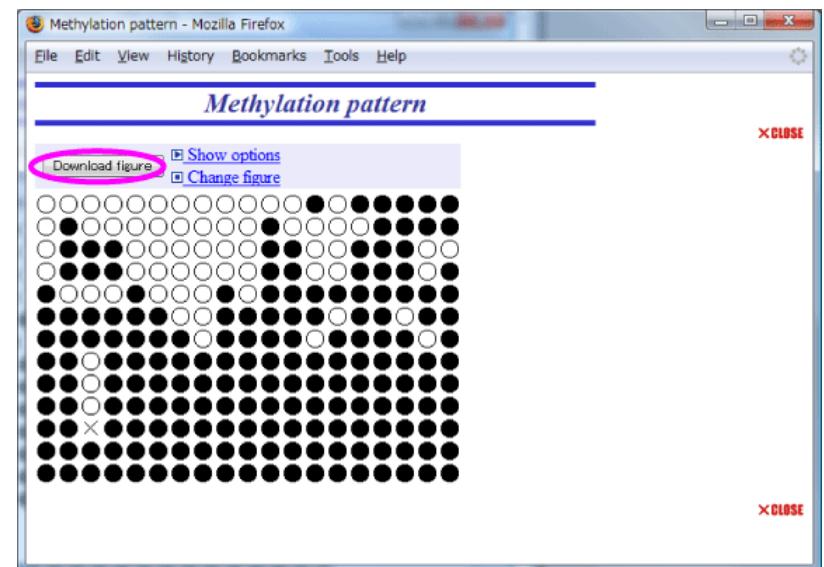
Downloaded alignment data file can be opened byTextEdit (Mac), Notepad (Win) or other text editors.



5.8. Figure page

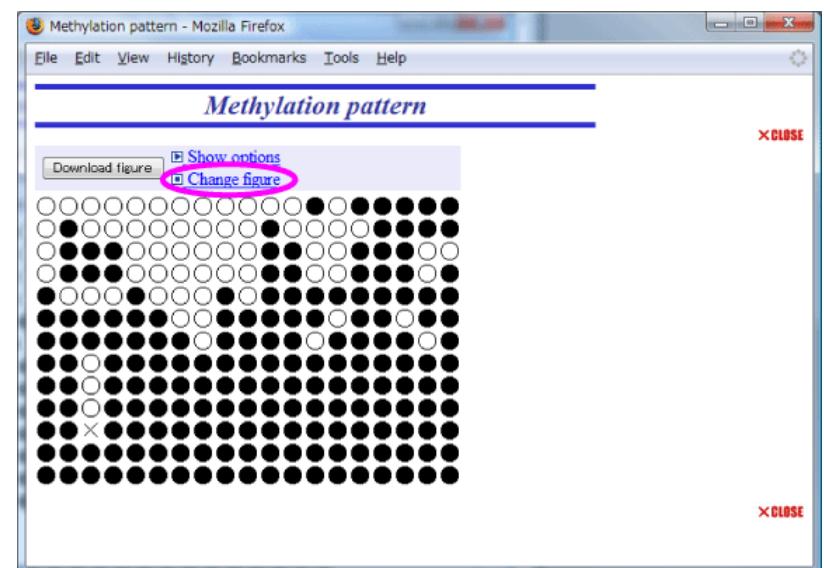
5.8.1. Download methylation pattern figure

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



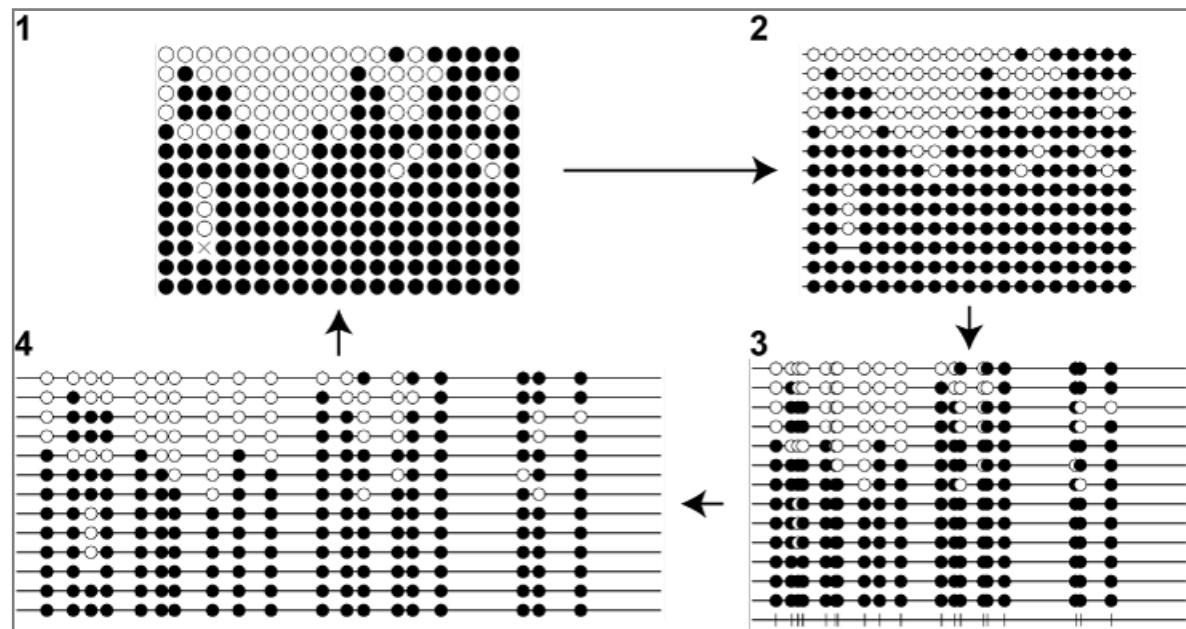
5.8.2. Change methylation pattern figure 1

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



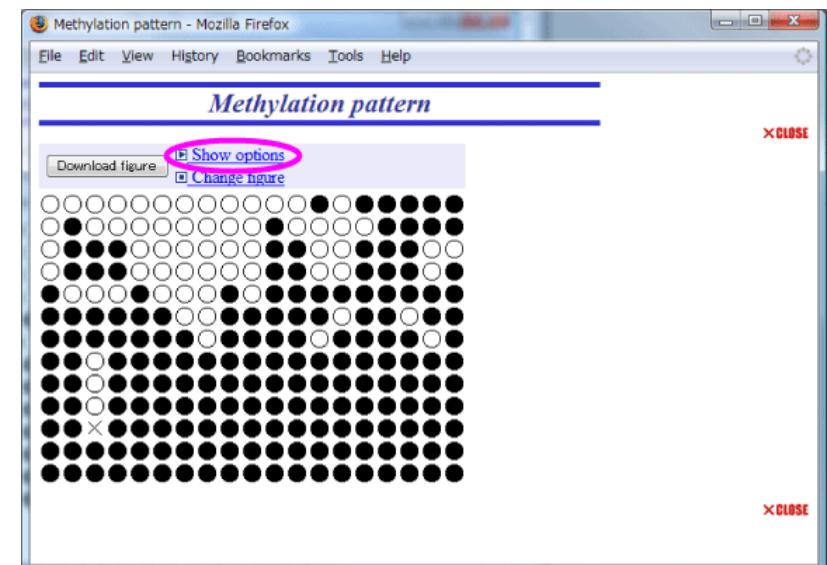
5.8.3. Change methylation pattern figure 2

Methylation pattern figures are switched one after the other.



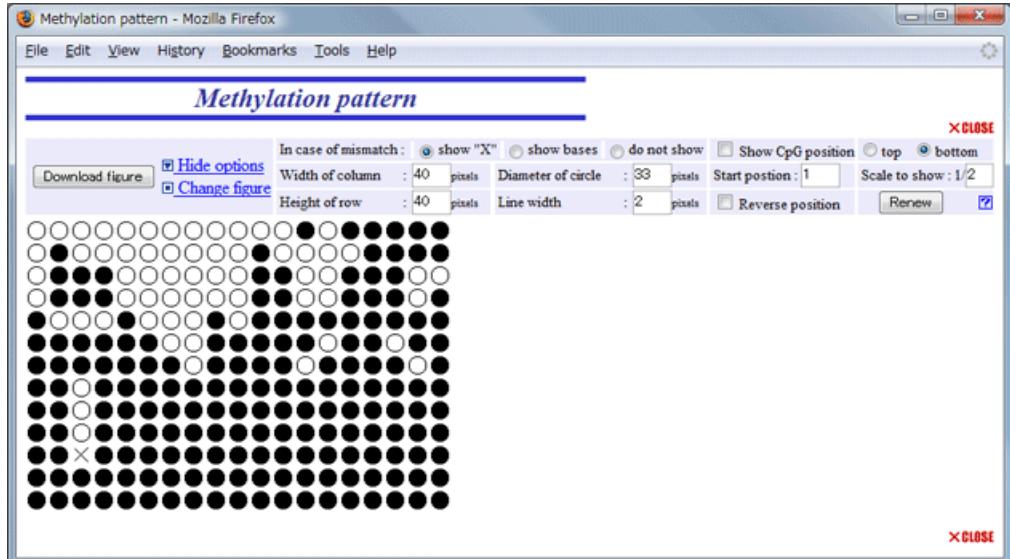
5.8.4. Show options

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



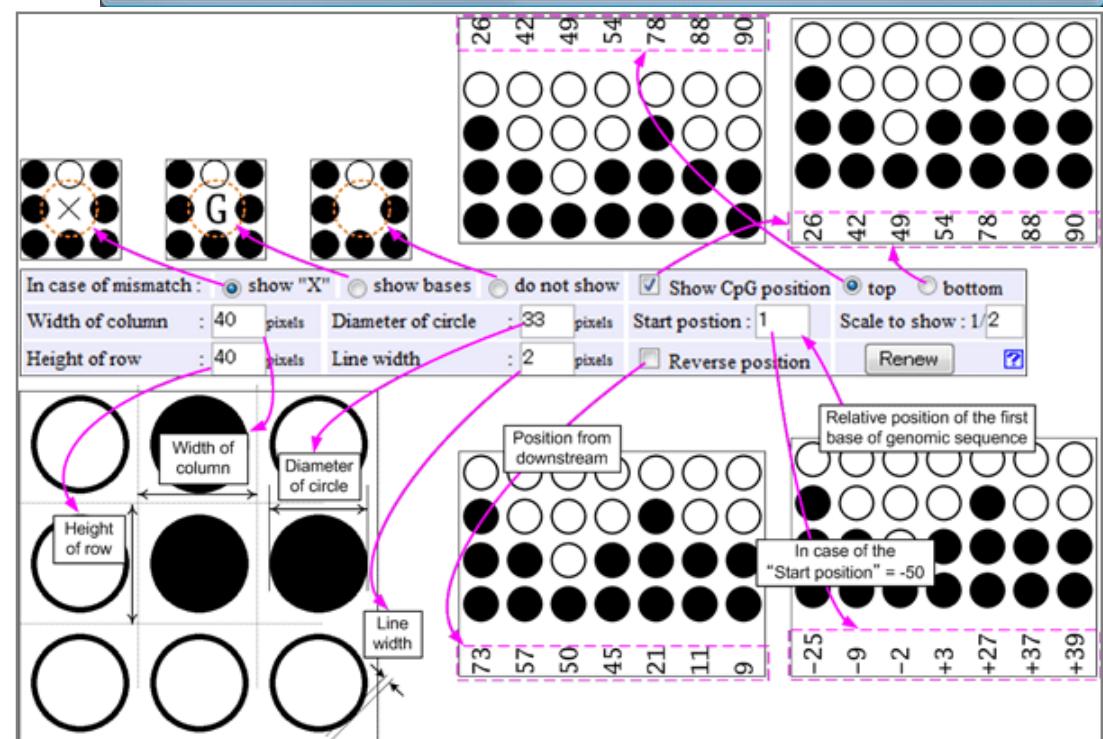
5.8.5. Figure 1

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.



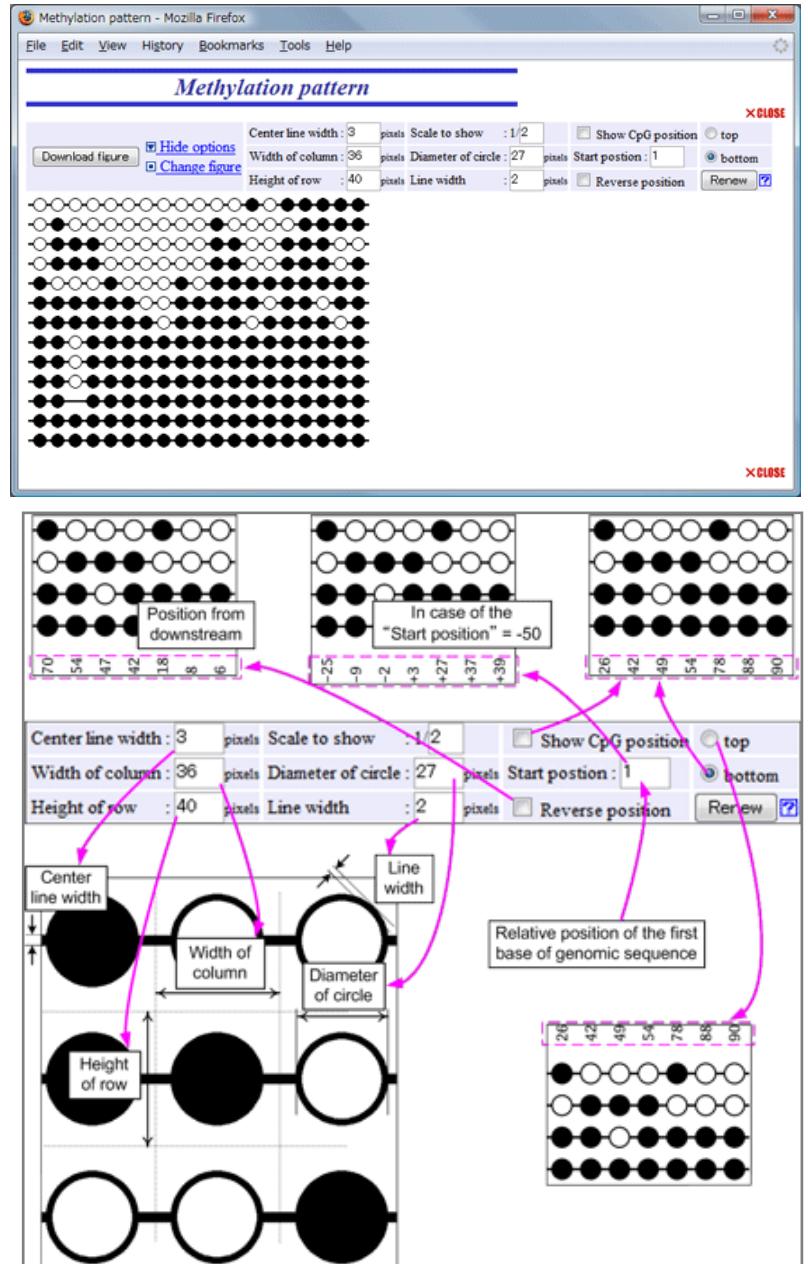
5.8.7. Figure 2

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

s

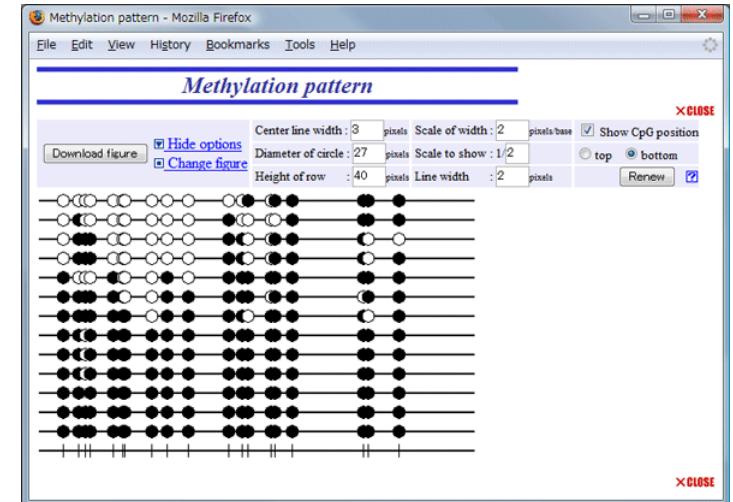
5.8.8. Option of figure 2

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.



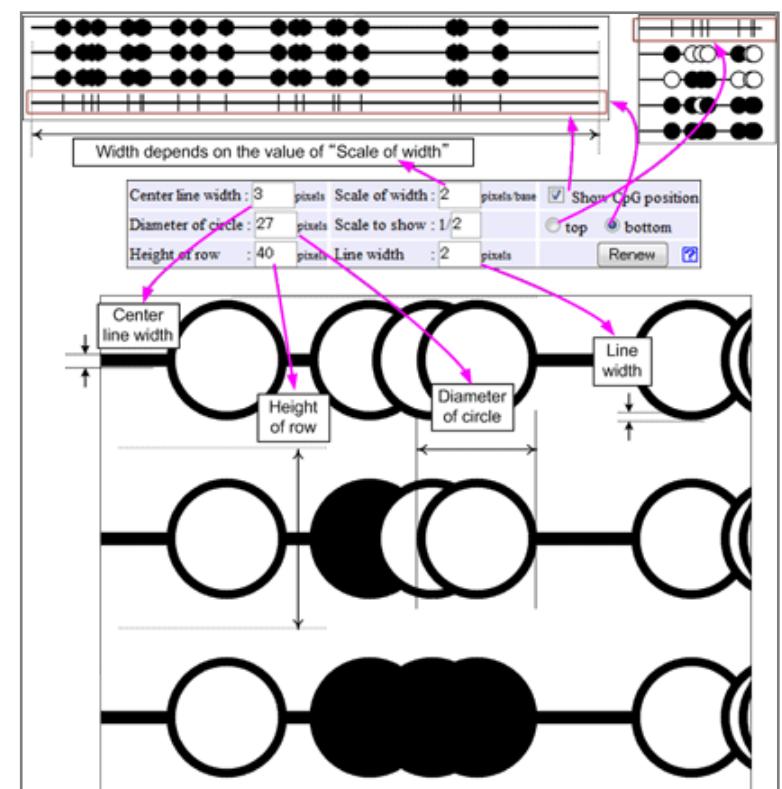
5.8.9. Figure 3

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.

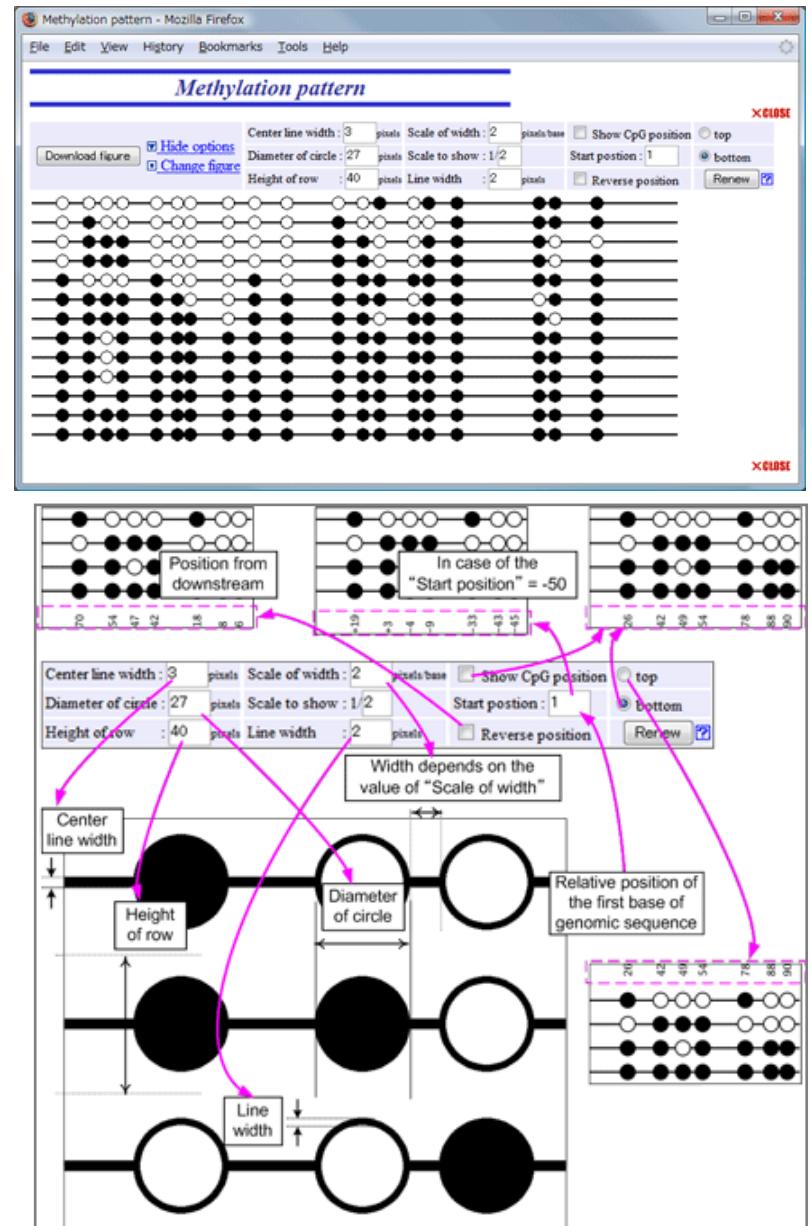


5.8.11. Figure 4

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.

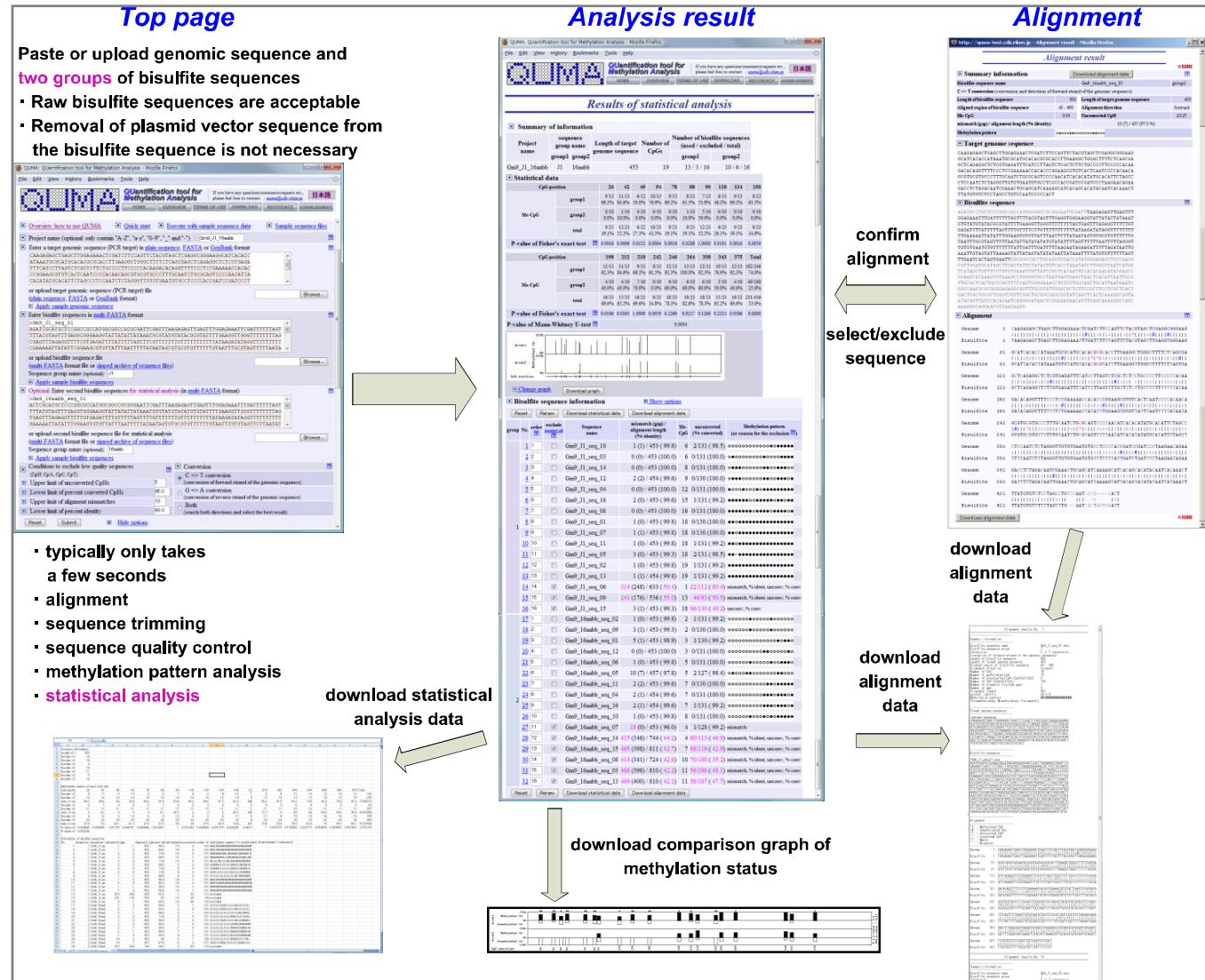


6. Statistical analysis mode

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 bottom center, there is a link labeled "Show options" with a red oval drawn around it. Other visible links include "Overview: how to use QUMA", "Quick start", "Execute with sample sequence data", and "Sample sequence files". There are also input fields for uploading target genomic sequences and bisulfite sequence files, along with instructions and checkboxes for these uploads.

This screenshot shows the QUMA homepage with the "Show options" link activated. The interface has expanded to reveal several additional input fields and checkboxes. These include:

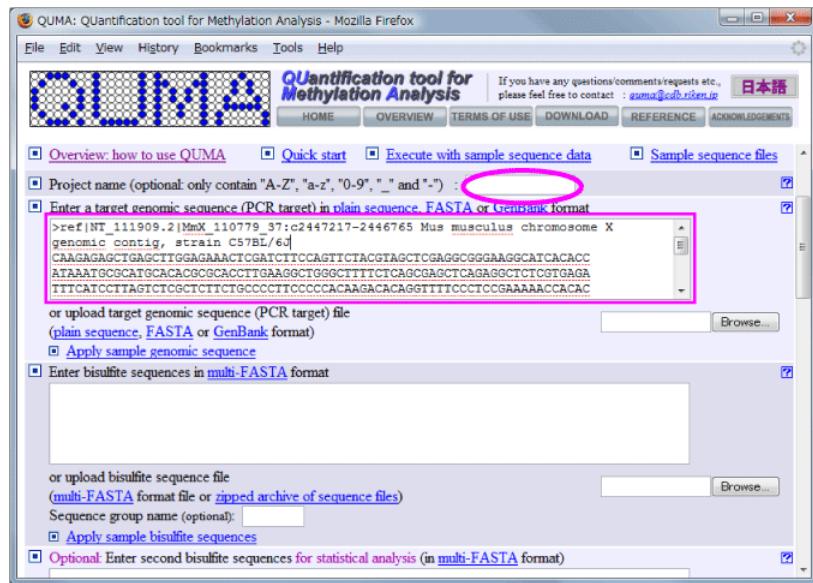
- Checkboxes for "Overview: how to use QUMA", "Quick start", "Execute with sample sequence data", and "Sample sequence files".
- A section for uploading target genomic sequences (PCR target) in plain sequence, FASTA, or GenBank format.
- A section for entering a target genomic sequence (PCR target) in plain sequence, FASTA, or GenBank format.
- A section for uploading bisulfite sequence files in multi-FASTA format.
- A section for entering bisulfite sequences in multi-FASTA format.
- A section for optional second bisulfite sequences for statistical analysis in multi-FASTA format.
- Conditions to exclude low quality sequences (CpI: CpA, CpC, CpT).
- 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).
- Conversion options: C => T conversion (conversion of forward strand of the genomic sequence), G => A conversion (conversion of reverse strand of the genomic sequence), and Both (search both directions and select the best result).

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.

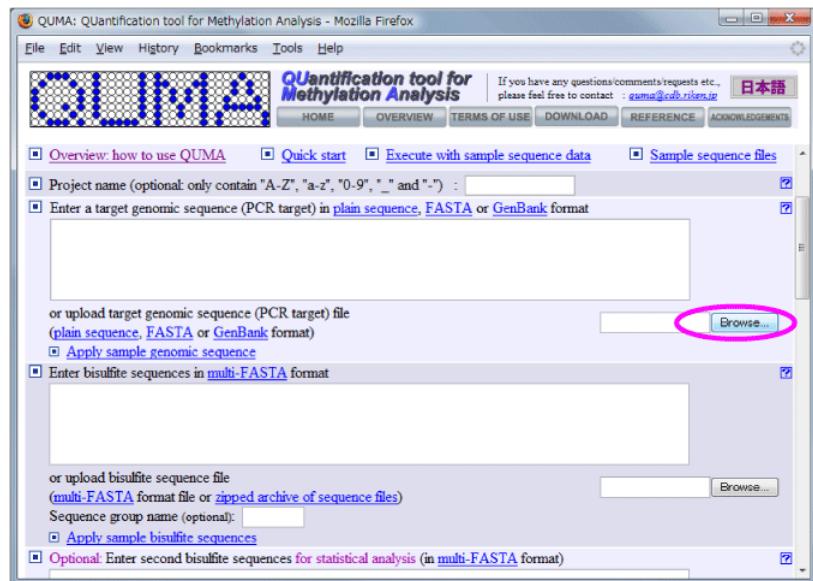
The target genomic sequence can be input by two ways of 1) direct input and 2) upload.

- 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](#)".



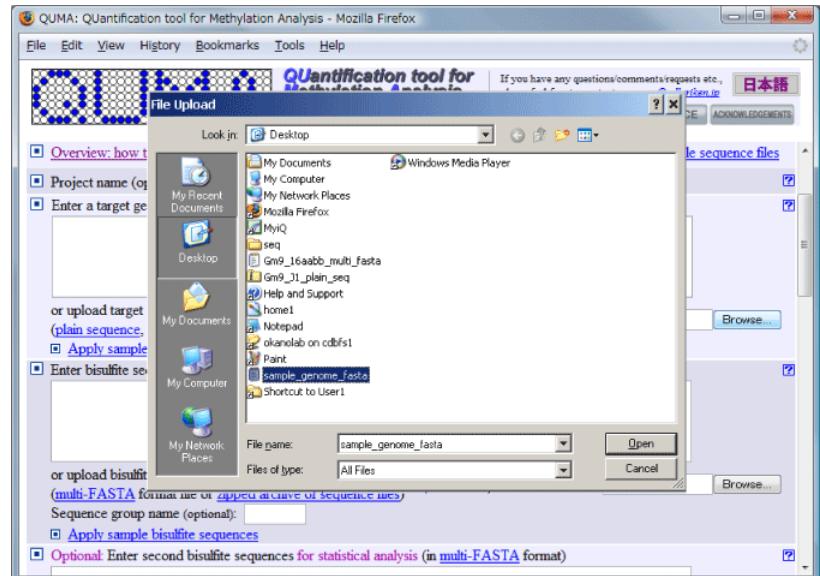
6.2.4. Genomic sequence file 1

- 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

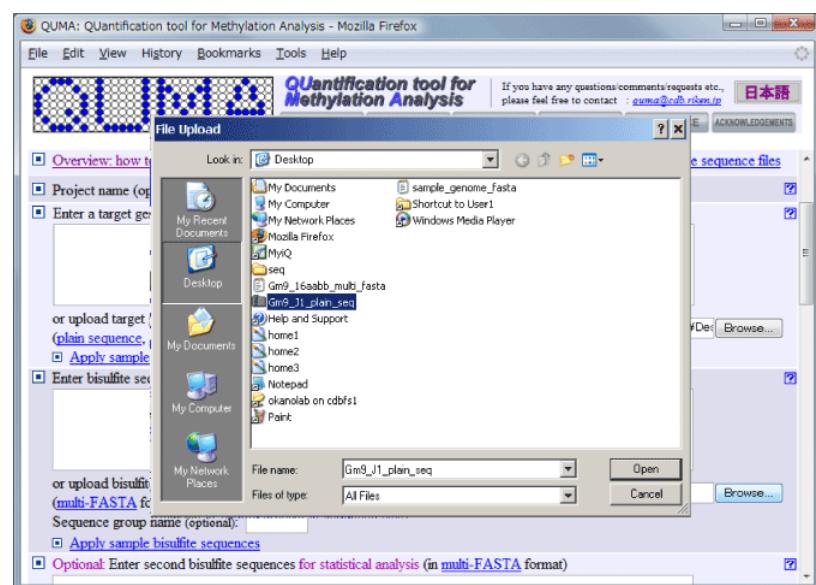
2) Or click the second button to upload a file of bisulfite sequences of first group.



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.



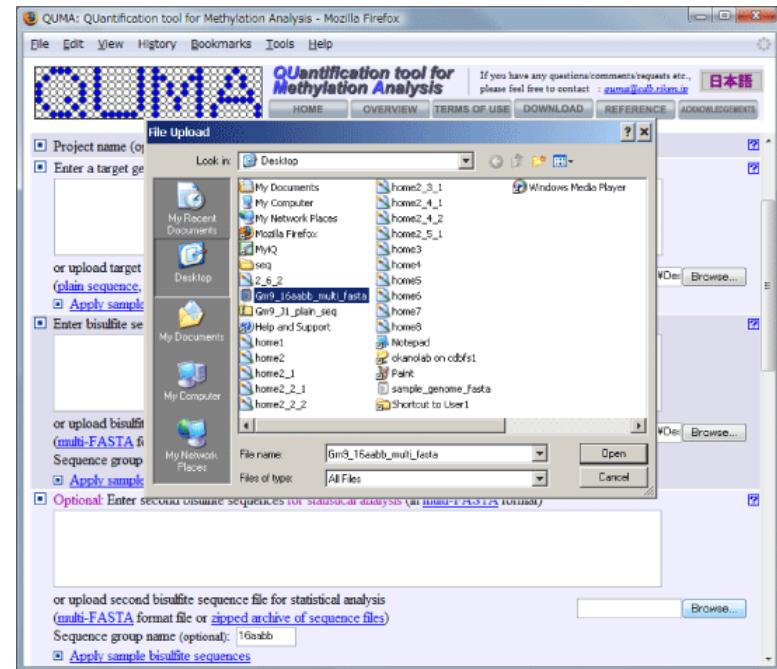
6.2.10. File of second bisulfite sequence group 1

- 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

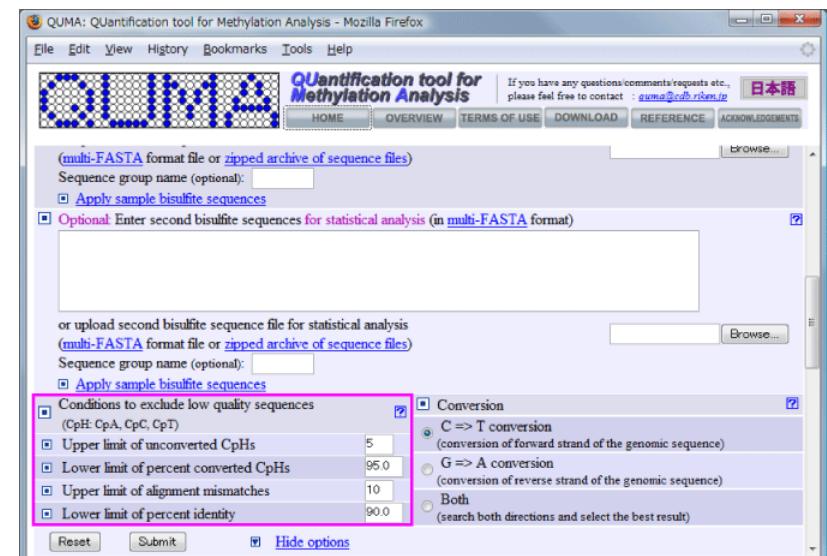
Select a file of bisulfite sequences of second group. The sequence file format of the second group is same as the first group.



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 screenshot shows the QUMA web interface. In the 'Conversion' section, the radio button for 'C => T conversion (conversion of forward strand of the genomic sequence)' is selected. Other options include 'G => A conversion (conversion of reverse strand of the genomic sequence)' and 'Both (search both directions and select the best result)'. There are also input fields for 'Upper limit of unconverted CpHs' (5), 'Lower limit of percent converted CpHs' (95.0), 'Upper limit of alignment mismatches' (10), and 'Lower limit of percent identity' (90.0). Buttons for 'Reset', 'Submit', and 'Hide options' are at the bottom.

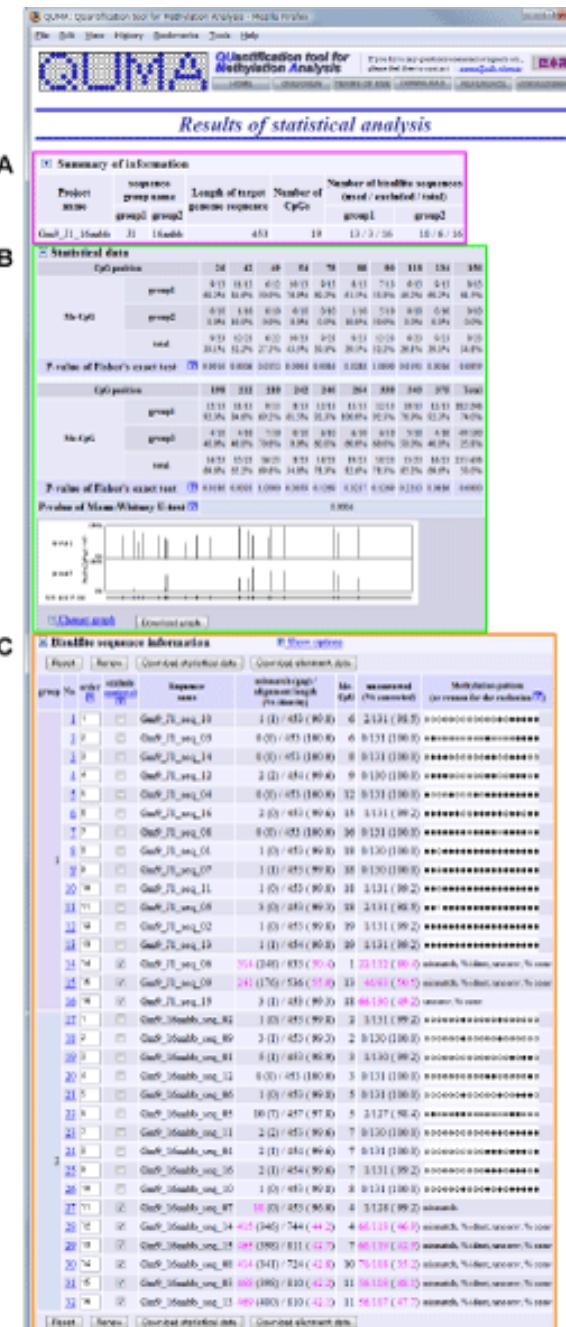
This screenshot is identical to the one above, showing the QUMA web interface with the 'C => T conversion' option selected. However, the 'Submit' button at the bottom left of the form is now highlighted with a pink circle.

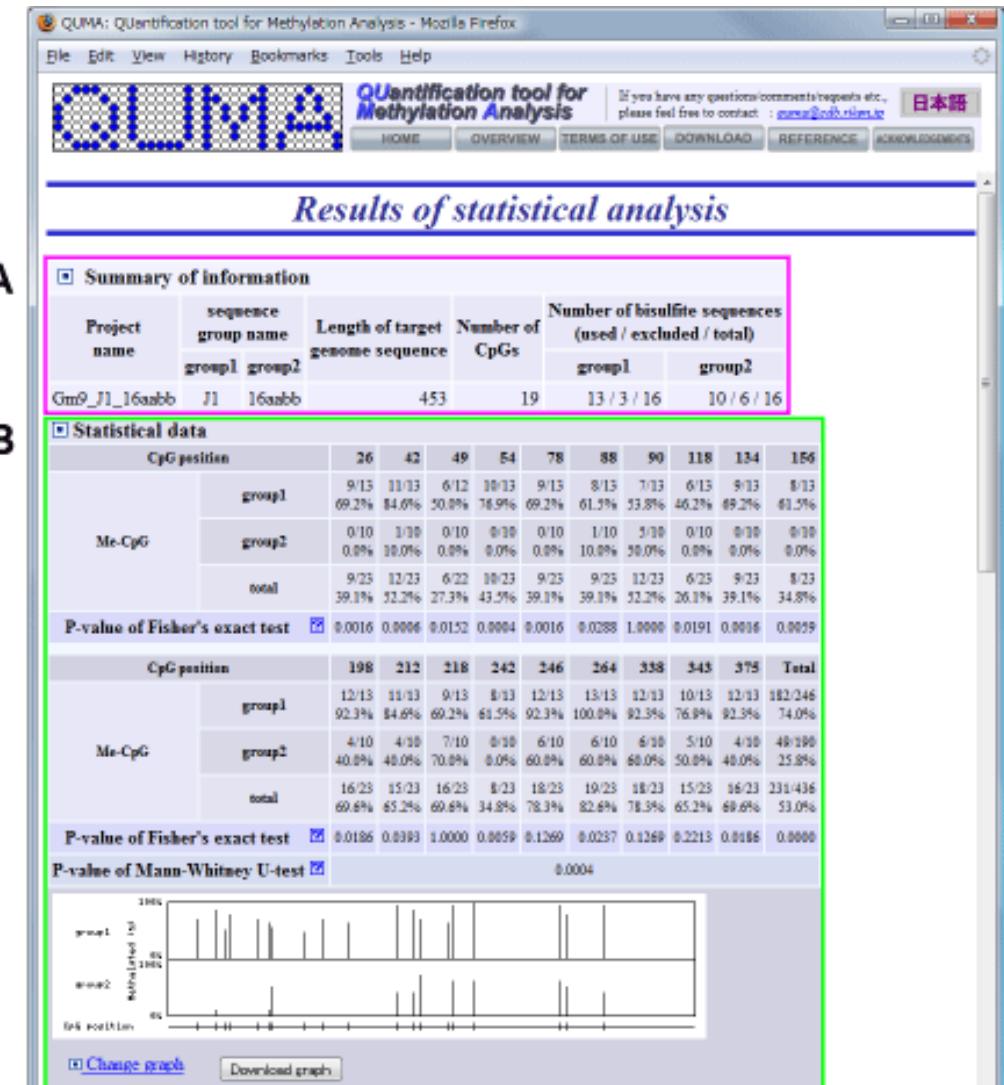
6.3. Statistical analysis result page

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.

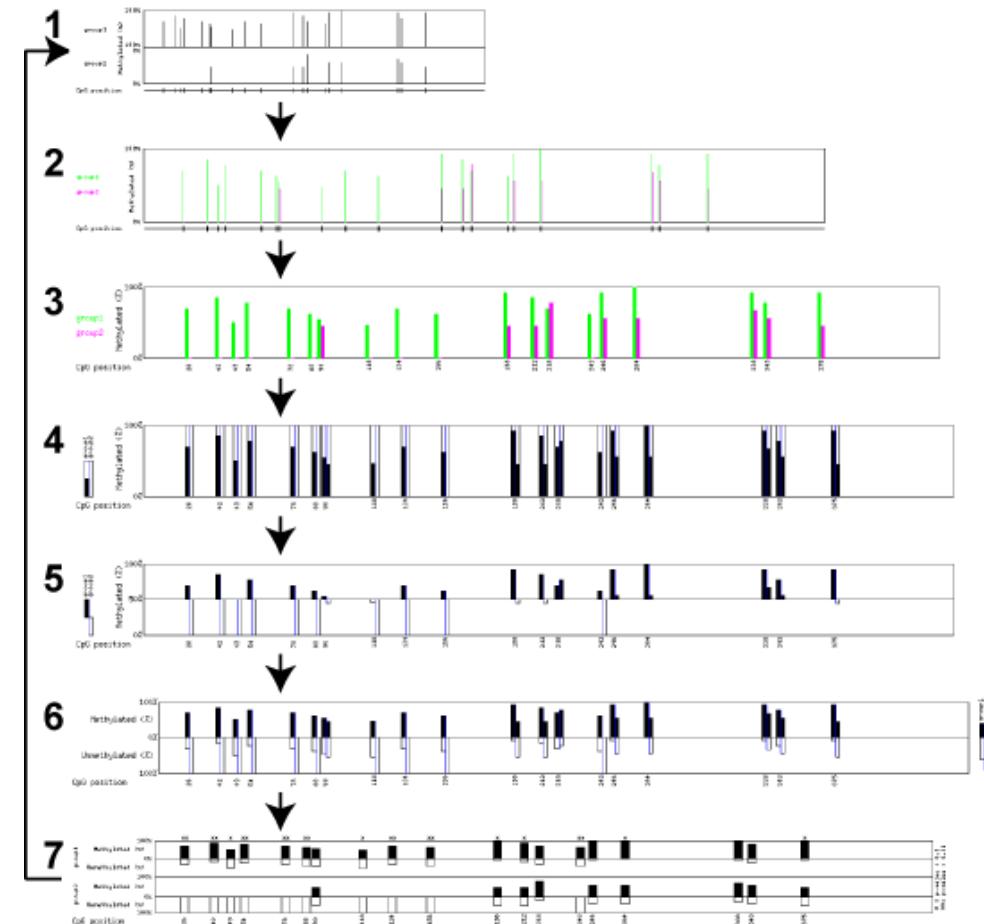
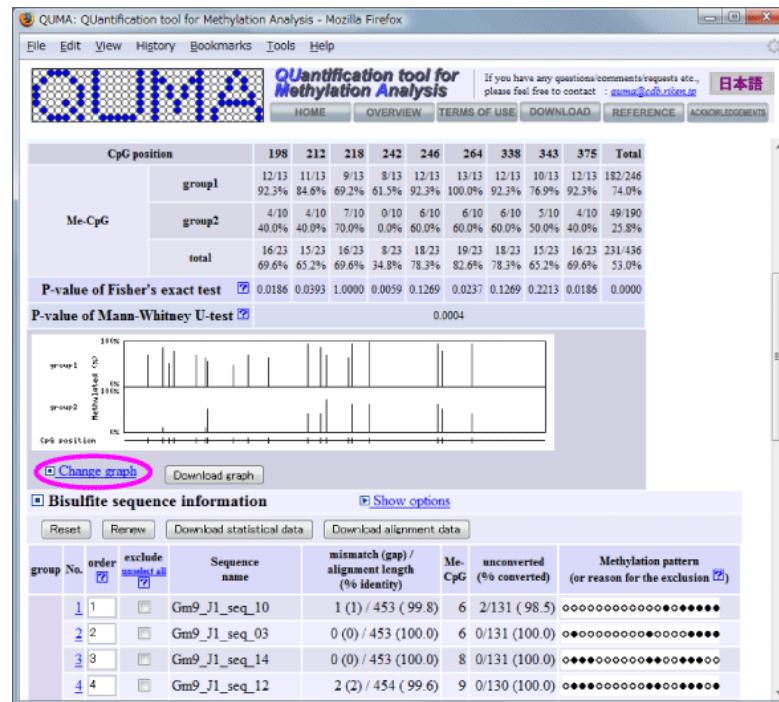
Mann-Whitney U-test: The statistical significance between two groups of the entire set of CpG sites is evaluated with the Mann-Whitney U-test (also called the Wilcoxon rank-sum test) that is non-parametric statistical significance test for two distributed samples. See "[9.2. Mann-Whitney U-test](#)"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

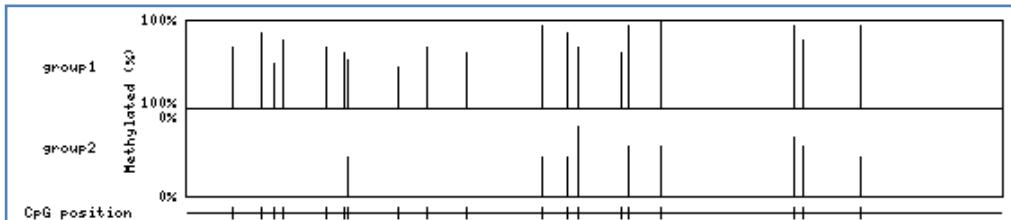
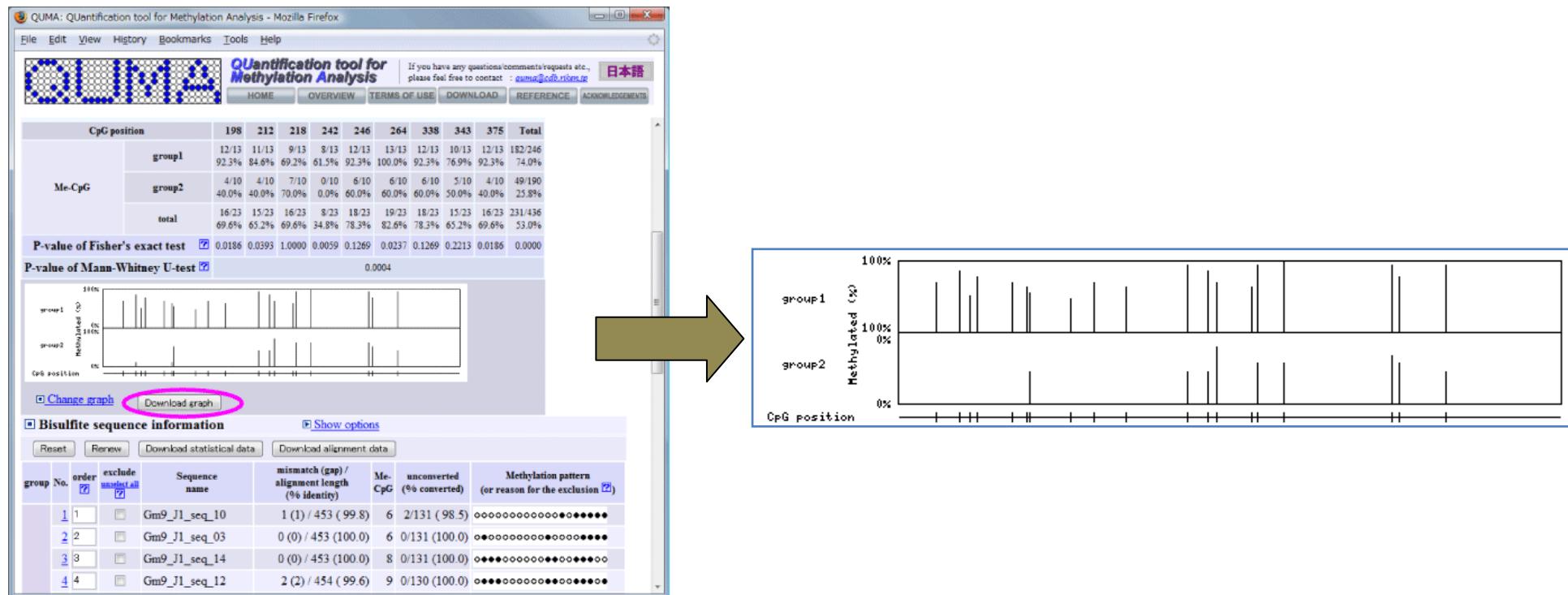


6.3.4. Change methylation status figure 2

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.



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).

C

Bisulfite sequence information [Show options](#)

group	No.	order	exclude mismatch 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	2		Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	ooooooooooooo•oooooo
	3	3		Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooo•oooooo
	4	4		Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooo
	5	5		Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooooo
	6	6		Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	oooooooooooooooooo
	7	7		Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooo
	8	8		Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	•oooooooooooooo
	9	9		Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooo
	10	10		Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooo
	11	11		Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	••xoooooooooooo
	12	12		Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooo
	13	13		Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooo
	14	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, % 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)	oooooooo•ooooooooo
	18	2		Gm9_16aabb_seq_09	3 (1) / 453 (99.3)	2	0/130 (100.0)	oooooooo•ooooooooo
	19	3		Gm9_16aabb_seq_01	5 (1) / 453 (98.9)	3	1/130 (99.2)	oooooooooooooooooo
	20	4		Gm9_16aabb_seq_12	0 (0) / 453 (100.0)	3	0/131 (100.0)	oooooooooooooooooo
	21	5		Gm9_16aabb_seq_06	1 (0) / 453 (99.8)	5	0/131 (100.0)	oooooooooooooooooo
	22	6		Gm9_16aabb_seq_05	10 (7) / 457 (97.8)	5	2/127 (98.4)	oooooooooooooooooo
	23	7		Gm9_16aabb_seq_11	2 (2) / 453 (99.6)	7	0/130 (100.0)	oooooooooooooooooo
	24	8		Gm9_16aabb_seq_04	2 (1) / 454 (99.6)	7	0/131 (100.0)	oooooooooooooooooo
	25	9		Gm9_16aabb_seq_16	2 (1) / 454 (99.6)	7	1/131 (99.2)	oooooooooooooooooo
	26	10		Gm9_16aabb_seq_10	1 (0) / 453 (99.8)	8	0/131 (100.0)	oooooooo•oooooooo
	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 unselect all	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG (% converted)	unconverted	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)	ooooooooooooooooooo
3	3	<input type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	ooooooooooooooooooo
4	4	<input type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	ooooooooooooooooooo
5	5	<input type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	ooooooooooooooooooo
6	6	<input type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	ooooooooooooooooooo
7	7	<input type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	ooooooooooooooooooo
8	8	<input type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooooooooo
9	9	<input type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	ooooooooooooooooooo
10	10	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	ooooooooooooooooooo
11	11	<input type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	ooooooooooooooooooo
12	12	<input type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	ooooooooooooooooooo
13	13	<input type="checkbox"/>	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
17	1	<input type="checkbox"/>	Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	ooooooooooooooooooo
18	2	<input type="checkbox"/>	Gm9_16aabb_seq_09	3 (1) / 453 (99.3)	2	0/130 (100.0)	ooooooooooooooooooo
19	3	<input type="checkbox"/>	Gm9_16aabb_seq_01	5 (1) / 453 (98.9)	3	1/130 (99.2)	ooooooooooooooooooo
20	4	<input type="checkbox"/>	Gm9_16aabb_seq_12	0 (0) / 453 (100.0)	3	0/131 (100.0)	ooooooooooooooooooo
21	5	<input type="checkbox"/>	Gm9_16aabb_seq_06	1 (0) / 453 (99.8)	5	0/131 (100.0)	ooooooooooooooooooo
22	6	<input type="checkbox"/>	Gm9_16aabb_seq_11	2 (2) / 453 (99.6)	7	0/130 (100.0)	ooooooooooooooooooo
23	7	<input type="checkbox"/>	Gm9_16aabb_seq_04	2 (1) / 454 (99.6)	7	0/131 (100.0)	ooooooooooooooooooo
24	8	<input type="checkbox"/>	Gm9_16aabb_seq_16	2 (1) / 454 (99.6)	7	1/131 (99.2)	ooooooooooooooooooo
25	9	<input type="checkbox"/>	Gm9_16aabb_seq_10	1 (0) / 453 (99.8)	8	0/131 (100.0)	ooooooooooooooooooo
26	10	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_07	18 (0) / 453 (96.0)	4	1/128 (99.2)	mismatch
27	11	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_14	415 (346) / 744 (44.2)	4	60/113 (46.9)	mismatch, % ident, unconv, % conv
28	12	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_05	10 (7) / 457 (97.8)	5	2/127 (98.4)	mismatch
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

group	order	exclude unselect all	Sequence name	mismatch (gap) / alignment length (% identity)	Me-CpG (% converted)	unconverted	Methylation pattern (or reason for the exclusion)
1	1	<input checked="" type="checkbox"/>	Gm9_J1_seq_12	1 (1) / 453 (99.8)	1	1/121 (99.4)	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
17	1	<input type="checkbox"/>	Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	ooooooooooooooooooo
18	2	<input type="checkbox"/>	Gm9_16aabb_seq_09	3 (1) / 453 (99.3)	2	0/130 (100.0)	ooooooooooooooooooo
19	3	<input type="checkbox"/>	Gm9_16aabb_seq_01	5 (1) / 453 (98.9)	3	1/130 (99.2)	ooooooooooooooooooo
20	4	<input type="checkbox"/>	Gm9_16aabb_seq_12	0 (0) / 453 (100.0)	3	0/131 (100.0)	ooooooooooooooooooo
21	5	<input type="checkbox"/>	Gm9_16aabb_seq_06	1 (0) / 453 (99.8)	5	0/131 (100.0)	ooooooooooooooooooo
22	6	<input type="checkbox"/>	Gm9_16aabb_seq_11	2 (2) / 453 (99.6)	7	0/130 (100.0)	ooooooooooooooooooo
23	7	<input type="checkbox"/>	Gm9_16aabb_seq_04	2 (1) / 454 (99.6)	7	0/131 (100.0)	ooooooooooooooooooo
24	8	<input type="checkbox"/>	Gm9_16aabb_seq_16	2 (1) / 454 (99.6)	7	1/131 (99.2)	ooooooooooooooooooo
25	9	<input type="checkbox"/>	Gm9_16aabb_seq_10	1 (0) / 453 (99.8)	8	0/131 (100.0)	ooooooooooooooooooo
26	10	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_07	18 (0) / 453 (96.0)	4	1/128 (99.2)	mismatch
27	11	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_14	415 (346) / 744 (44.2)	4	60/113 (46.9)	mismatch, % ident, unconv, % conv
28	12	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_05	10 (7) / 457 (97.8)	5	2/127 (98.4)	mismatch
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

6.3.9. Include/exclude bisulfite sequence 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)
13	14	<input checked="" type="checkbox"/>	Gm9_J1_seq_06	314 (248) / 633 (50.4)	1	22/112 (80.4)	mismatch, % ident, unconv, % conv
14	15	<input checked="" type="checkbox"/>	Gm9_J1_seq_09	241 (176) / 536 (55.0)	13	46/93 (50.5)	mismatch, % ident, unconv, % conv
15	16	<input checked="" type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, % conv
16	1	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_02	1 (0) / 453 (99.8)	2	1/131 (99.2)	oooooooooooooooooooo
17	2	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_09	3 (1) / 453 (99.3)	2	0/130 (100.0)	oooooooooooooooooooo
18	3	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_01	5 (1) / 453 (98.9)	3	1/130 (99.2)	oooooooooooooooooooo
19	4	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_12	0 (0) / 453 (100.0)	3	0/131 (100.0)	oooooooooooooooooooo
20	5	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_06	1 (0) / 453 (99.8)	5	0/131 (100.0)	oooooooooooooooooooo
21	6	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_11	2 (2) / 453 (99.6)	7	0/130 (100.0)	oooooooooooooooooooo
22	7	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_04	2 (1) / 454 (99.6)	7	0/131 (100.0)	oooooooooooooooooooo
23	8	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_16	2 (1) / 454 (99.6)	7	1/131 (99.2)	oooooooooooooooooooo
24	9	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_10	1 (0) / 453 (99.8)	8	0/131 (100.0)	oooooooooooooooooooo
25	10	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_07	18 (0) / 453 (96.0)	4	1/128 (99.2)	oooooooooooooooooooo
26	11	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_05	10 (7) / 457 (97.8)	5	2/127 (98.4)	oooooooooooooooooooo
27	12	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_14	415 (346) / 744 (44.2)	4	60/113 (46.9)	mismatch, % ident, unconv, % conv
28	13	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_15	465 (398) / 811 (42.7)	7	68/119 (42.9)	mismatch, % ident, unconv, % conv
29	14	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_08	414 (341) / 724 (42.8)	10	70/108 (35.2)	mismatch, % ident, unconv, % conv
30	15	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_03	468 (398) / 810 (42.2)	11	56/108 (48.1)	mismatch, % ident, unconv, % conv
31	16	<input checked="" type="checkbox"/>	Gm9_16aabb_seq_13	469 (400) / 810 (42.1)	11	56/107 (47.7)	mismatch, % ident, unconv, % conv

6.3.10. Change the order of bisulfite sequences 1

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

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

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*ooooooooooooo

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)	*****ooooooooooooo*****
3			Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	*****ooooooooooooo*****
4			Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	*****ooooooooooooo*****
5			Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	*****ooooooooooooo*****
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*ooooooooooooo

6.3.13. Alignments data

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



The screenshot shows the QUMA software interface with the title "QUMA: Quantification tool for Methylation Analysis - Mozilla Firefox". The main window displays a table titled "Bisulfite sequence information". The table has columns for group, No., order, exclude, sequence name, mismatch (gap), alignment length (% identity), Me CpG, unconverted (% converted), and Methylation pattern (or reason for the exclusion). A red circle highlights the "Download statistical data" button at the top of the table.

6.3.14. Download statistical analysis data

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

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](#)".

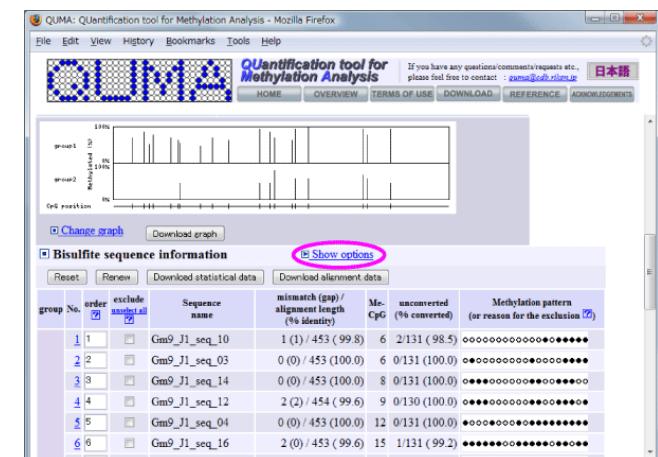
The screenshot shows a Microsoft Excel spreadsheet with data from a CSV file. The data includes:

- Summary Information: Length of 1, Number of 19, Number of 13, Number of 3, Number of 16, Number of 5, Number of 11, Number of 5, Number of 16.
- Methylation status of each CpG site: Data for 12 CpG positions across 17 samples.
- P-value of 0.000589, 0.000644, 0.01373, 0.000151, 0.000589, 0.013067, 1, 0.016182, 0.000589, 0.000174, 0.023265, 0.06247, 1, 0.00174, 0.0126899, 0.023715, 0.060804, 0.050452, 0.007807, 2.31E-25.
- Information of bisulfite sequence: Data for 54 samples, including sequence, mismatch, alignment, percent dimethylated, unconverted, and methylation patterns (U unmethylated, M methylated, X mismatch).

6.4. Statistical analysis result page options

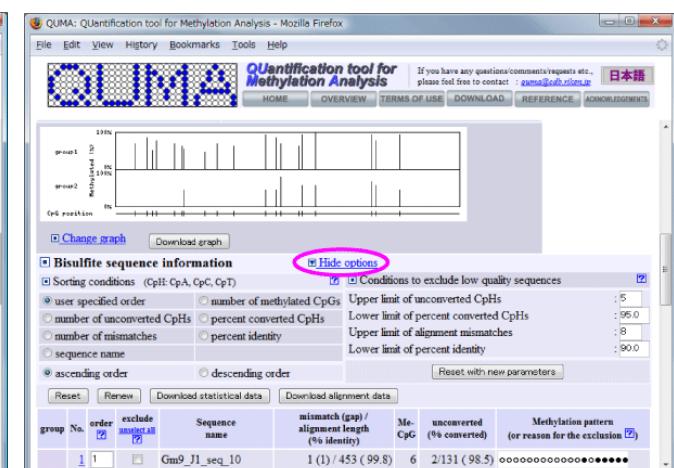
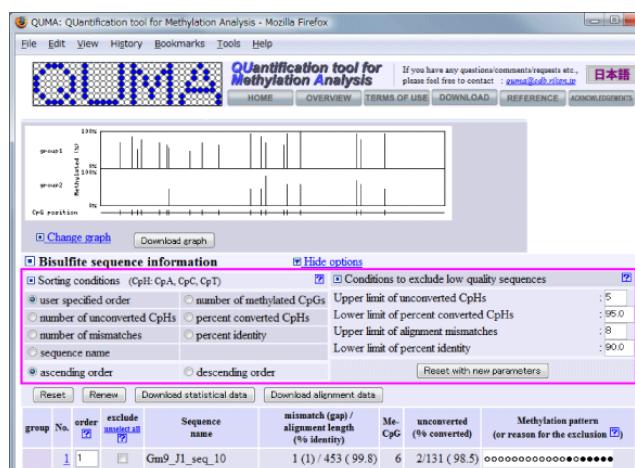
6.4.1. Show options 1

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).



6.4.4. 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)	ooooooocoooo*****
	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)	ooooooocoooo*****
	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	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_10	1 (1) / 453 (99.8)	6	2/131 (98.5)	oooooooooooooooooooo
	2	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
	3	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
	4	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooo
	5	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooo
	6	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	*****oo*****oooo
	7	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
	8	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
	9	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
	10	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
	11	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oo*****oooooooo
	12	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
	13	<input type="checkbox"/>	<input checked="" 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	22/112 (80.5)	mismatch, %ident, unconv, % conv
	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	<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	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_03	0 (0) / 453 (100.0)	6	0/131 (100.0)	oooooooooooooooooooo
	2	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_14	0 (0) / 453 (100.0)	8	0/131 (100.0)	oooooooooooooooooooo
	3	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_12	2 (2) / 454 (99.6)	9	0/130 (100.0)	oooooooooooooooooooo
	4	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_04	0 (0) / 453 (100.0)	12	0/131 (100.0)	oooooooooooooooo
	5	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_16	2 (0) / 453 (99.6)	15	1/131 (99.2)	*****oo*****oooo
	6	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_08	0 (0) / 453 (100.0)	16	0/131 (100.0)	oooooooooooooooooooo
	7	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_01	1 (0) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
	8	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_07	1 (1) / 453 (99.8)	18	0/130 (100.0)	oooooooooooooooooooo
	9	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
	10	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_05	3 (0) / 453 (99.3)	18	2/131 (98.5)	oo*****oooooooo
	11	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Gm9_J1_seq_02	1 (0) / 453 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
	12	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_13	1 (1) / 454 (99.8)	19	1/131 (99.2)	oooooooooooooooooooo
	13	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_11	1 (0) / 453 (99.8)	18	1/131 (99.2)	oooooooooooooooooooo
	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	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Gm9_J1_seq_15	3 (1) / 453 (99.3)	18	66/130 (49.2)	unconv, % conv
	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.



6.5.2. Download alignment data

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

The screenshot shows the QUMA web interface with the following details:

- Summary Information:**
 - Bisulfite sequence name: Gm0_16abbb_seq_05
 - Bisulfite sequence group: group2
 - Conversion: C => T conversion
 - Length of bisulfite sequence: 983
 - Length of target genome sequence: 453
 - Aligned region of bisulfite sequence: 45 - 498
 - Unconverted CpH: 5/19
 - Alignment direction: forward
 - Number of CpG: 19
 - Number of methylated CpG: 5
 - Number of Unconverted CpH (CnA/CpT/CpC): 2
 - Number of CpH (CnA/CpT/CpC): 127
 - Number of mismatch (include gap): 10
 - Number of gap: 7
 - Number of mismatch: 10
 - Alignment length: 457
 - % identity: 97.8 %
 - Methylation pattern: UUUUMLUUUUUMLUUU (U unmethylated, M methylated, L mismatch)
- Target genome sequence:** A long sequence of DNA bases (A, T, C, G) is shown below the summary table.
- Alignment result:** A table showing the alignment between the bisulfite sequence and the target genome sequence. It includes columns for Length of bisulfite sequence, Length of target genome sequence, Aligned region of bisulfite sequence, Alignment direction, Me-CpG, Unconverted CpH, mismatch (gap) / alignment length (% identity), and Methylation pattern.

6.5.3. Alignment data

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

7. Input data

7.1. Genomic sequence

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

Sequence of [8.1. Plain sequence](#), [8.2. FASTA](#) or [8.3. GenBank](#) format is acceptable. Only [rich text format \(with ".rtf" file extension\)](#) or [plain text format text file](#) is acceptable for upload file. [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  
GACCCGCGCGCGATCGCGGCCGCGCTACAGCCAGCCTACTGGCGC  
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  
CGCAGCCGCAGCCCGTGGACGCTCTCGCCTGAGCGCCGCGGACTAGCC  
CGGGTGGCCCAGTGGCGCGGGCGAGCGCACGGCGCTCCAGTCCGGCA  
GCGCCGGGTTAACGCGCCAAGTAAACGTAGCGCAGCGATCGCGCCGG  
AGATTCGCGAACCGACACTCCGCGCCGCCAGGACCCGCG  
GCGATCGCGCGCCCGCTACAGCCAGCCTCACGACAGGCCGCTGAGGC  
TTGTGCCAGACCTTGAAACCTCAGGTATATACTTCCAGACGGCGGGAT  
CTCCCCCTCCCCATCCATAGTGCCTTGGGACCAAATCCAGGGCCTCTTT  
CAGGAAACAATGAAGGGAGACAGCAGACATCTGAATGAAGAAGAGGGTGC  
CAGCGGGTATGAGGAGTCATTATCGTTAATGGAACTTCAGTGACCAGT  
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 Location/Qualifiers
source   1..200
          /organism="Mus musculus"
          /mol_type="mRNA"
          /db_xref="taxon:10090"
          /chromosome="12"
          /map="4.0 cM"
gene     1..>200
          /gene="Dnmt3a"
ORIGIN
       1 gaattccggc ctgctgccgg gcccggccgac 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
ACTCCCCGTGCGCGCCGGCCCGTAGCGTCCTCGCGCCCGTCTCGCAGCCGCA
GCCCGCGTGGACGCTCTGCCTGAGGCCGCGGACTAGCCGGTGGCC
>sequence2
CAGTCCGGCAGGCCGGGTTAACGCCCCAAGTAAACGTAGCGCAGCGATGGCGCCGG
AGATTCGCGAACCCGACACTCCCGCCGCCGCCAGGACCCGCGCGATCGCGG
CGCCGCGCTACAGCCAGCCTCACTGGCGCGGGCGAGCGCACGGCGCTC
>sequence3
CACGACAGGCCGCTGAGGCTTGTGCCAGACCTGGAAACCTCAGGTATATACTTCCA
GACGCGGGATCTCCCCTCCCC
>sequence4
CAGCAGACATCTGAATGAAGAAGAGGGTGCCAGCGGGTATGAGGAGTGCATTATCGTTAA
TGGGAACCTCAGTGACCAGTCCTCAGACACGAAGGATGCTCCCTACCCCCAGTCTTGA
GGCAATCTGCACAGAGCCAGTCTGCACACC
```

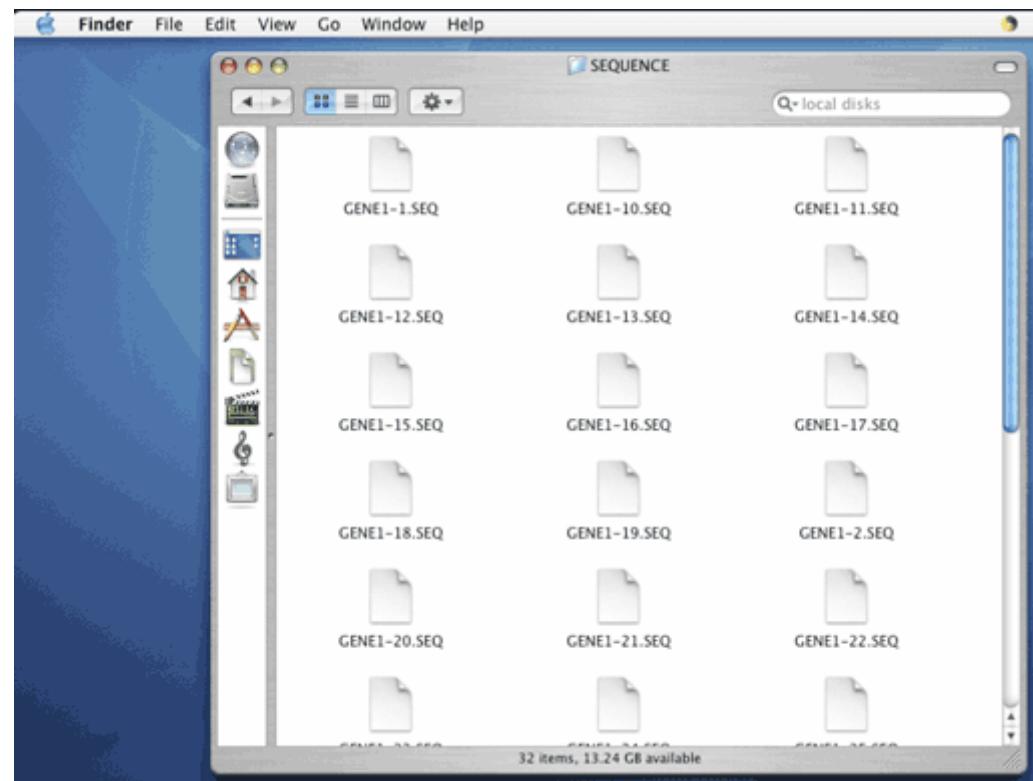
8.5. Zipped archive of sequence files

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".

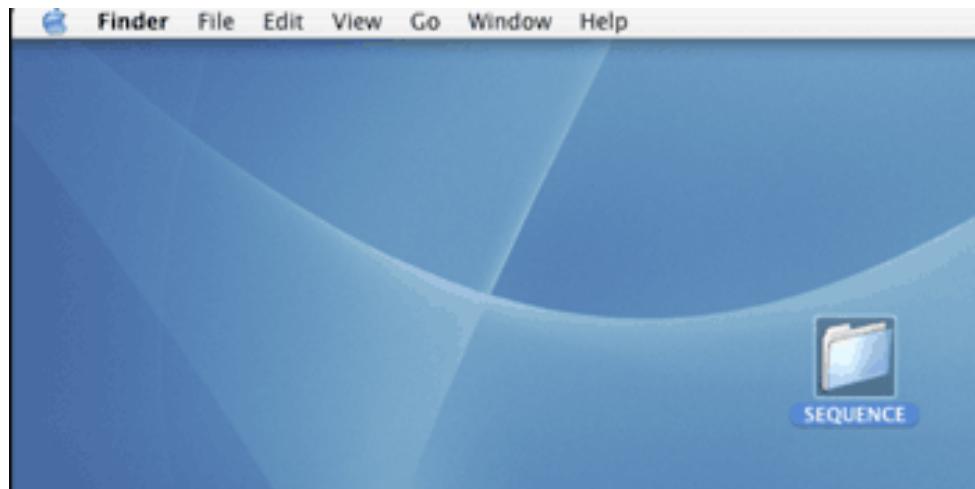
8.6. How to create zipped archive (Macintosh)

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.



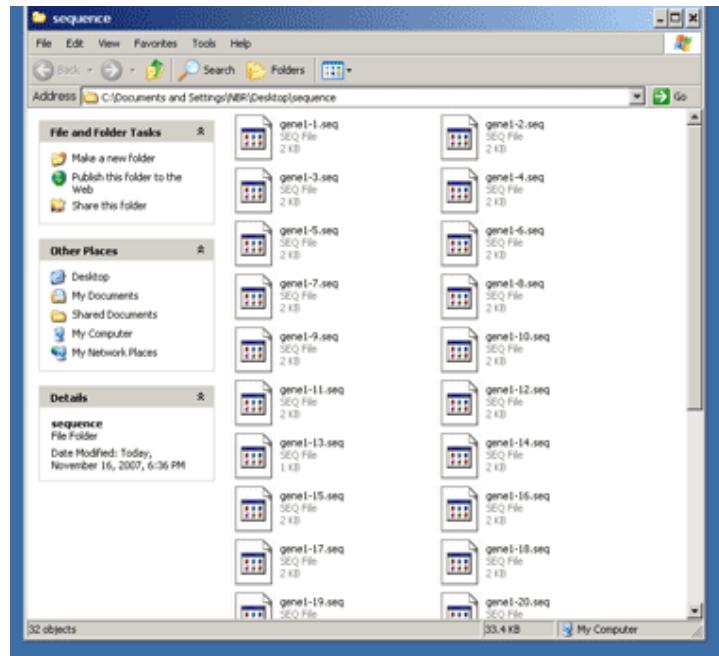
8.6.2. Other Mac OS

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

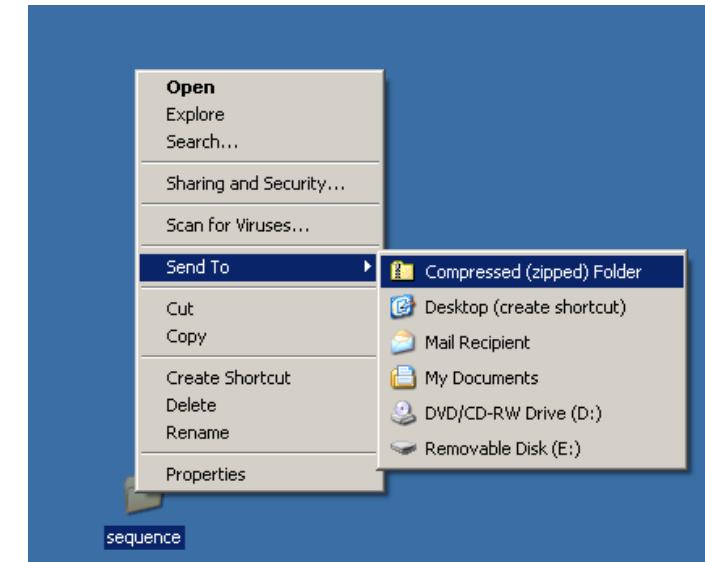
8.7. How to create zipped archive (Windows)

8.7.1. Windows Me/XP/Vista

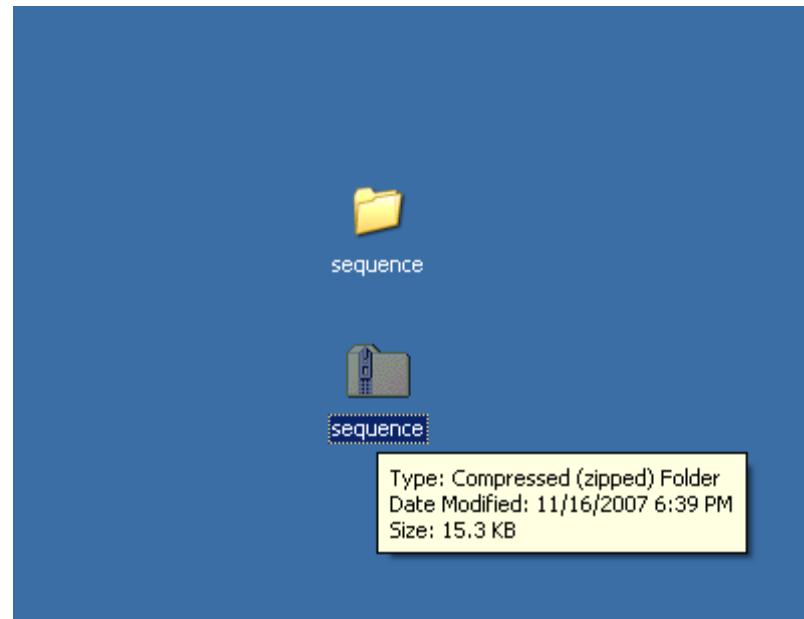
1. Put bisulfite sequence files of [8.2. FASTA](#) or [8.1. Plain sequence](#) format into a folder. (Acceptable file extension of sequence file is ".seq", ".fa", ".fas", ".fasta" or ".txt".)



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:

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

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): R_1 and R_2 .

Position i	1	2	3	4	5	6	7	8	9	10	11	12	Rank sum
Me-CpGs/CpGs	2/19	3/19	5/19	6/19	7/19	8/19	9/19	12/19	15/19	16/19	18/19	1	
rank	1,2	3,4	5,6	7,8	9-11	12,13	14	15	16	17	18-20	21-23	
rank (average)	1.5	3.5	5.5	7.5	10	12.5	14	15	16	17	19	22	
number of sequences	group1	0	0	0	2	0	1	1	1	1	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, U_1 and U_2 , as below.

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

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

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

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

The normal approximation is performed as:

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

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

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

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

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

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

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

Table 1

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

Table 2

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

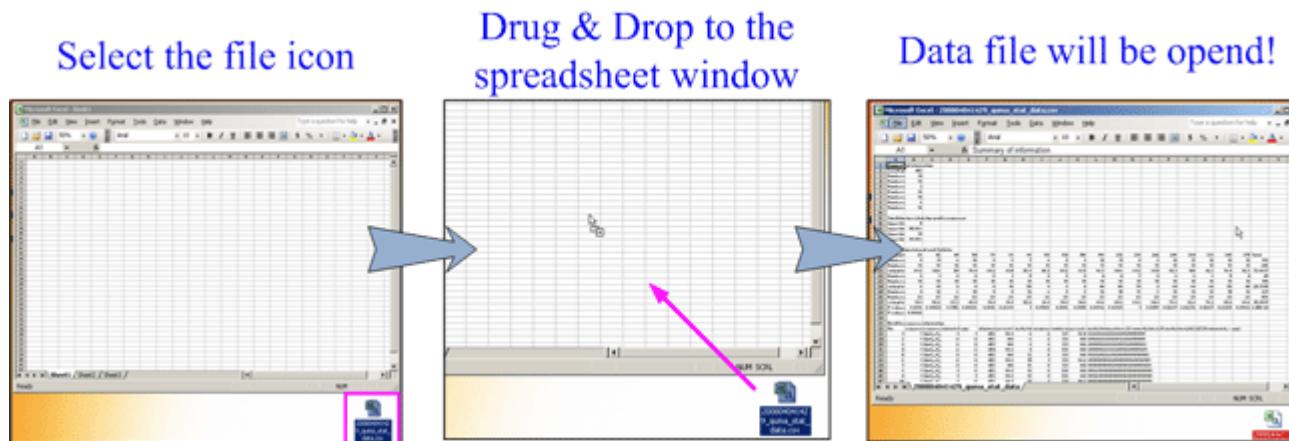
In many case, CSV formatted analysis data file can be opened from Microsoft Excel by double-clicking the file icon. If not, try the "drug & drop" procedure indicated below.

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").