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introduction

QTL-seq_GUI

QTL-seq_GUI は、異なる品種間交雑から得られた集団内で特定の表現型を示す個体に共通する遺伝子領域を、全ゲノムシーケンスデータに基づいて迅速に同定するためのツール。

本ツールに関する問題報告やご要望は、GitHub (https://github.com/sakemiki/QTL-seq_GUI) の「Issues」または「Pull Request」からご連絡ください。

preparation


Install the following tools:

Samtools (URL : <http://www.htslib.org/download/>)

Bcftools (URL : <http://www.htslib.org/download/>)

Python3 (URL : <https://www.python.org/downloads/>)

Reference fasta file
bam file

 Confirm that the .bam.bai file exists in the same directory as the bam file. If not, the process will stop.

Using the GUI

The screenshot displays the QTL-sq_GUI interface, which is organized into three main sections for user input:

- Enter the path to the file:** This section contains eight rows, each with a label, a text input field, and a 'Browse' button. The labels and their corresponding input values are: Output folder (/Users/xxxx/Desktop/output), samtools (/Users/xxxx/Desktop/samtools), bcftools (/Users/xxxx/Desktop/bcftools), fasta (/Users/xxxx/Desktop/reference.fa), P1 bam (/Users/xxxx/Desktop/P1.sort.bam), P2 bam (/Users/xxxx/Desktop/P2.sort.bam), F1 bam (/Users/xxxx/Desktop/F1.sort.bam), Abulk bam (/Users/xxxx/Desktop/Abulk.sort.bam), and Bbulk bam (/Users/xxxx/Desktop/Bbulk.sort.bam).
- Enter the name of bam and Enter filtering values:** This section contains two rows of input fields. The first row has fields for P1 name (P1), P2 name (P2), F1 name (F1), Abulk name (Abulk), and Bbulk name (Bbulk). The second row has fields for filter depth (10), window size (1000000), step size (50000), and bulk number (20).
- Select processing details:** This section contains four rows of radio button options. The first row has 'fai file' with 'Exists' selected and 'Not Exists' as an option. The second row has 'Bulk' with 'single' and 'double' as options, with 'double' selected. The third row has 'Bulk type' with 'RIL' and 'F2' as options, with 'F2' selected. The fourth row has 'pattern' with 'P1', 'P1, P2', 'P1, P2, F1' (selected), and 'P1, F1' as options.

A 'Run' button is located at the bottom right of the interface.

How to specify files and folders using the GUI in QTL-sq_GUI:

- Enter the path: the path of the file or folder can be entered directly in the input field.
- Using the Browse button: clicking on the 'Browse' button opens the file dialog.

Run.

Once the required options, such as 'output folder' and process commands, have been entered, execution can be started by clicking on the 'Run' button at the bottom right of the screen.

Using the GUI

The QTL-seq_GUI has the following three input fields.

1. Enter the path to the file

Enter the paths for the output folder, required tools, and the bam files to be used, or select them using the ' Browse ' button.

2. Enter the name of bam and Enter filtering values

Please enter the name of the bam file to be used and the numeric settings for the filter process.

3. Select processing details

Please select the appropriate option. ' pattern ' refers to the type of bam file used for filtering.


Run

1. Enter the path to the file :

Output folder	<input type="text" value="/Users/xxxx/Desktop/output"/>	<input type="button" value="Browse"/>
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Output Folder:

Select the folder where the results generated by QTL-seq_GUI are stored.

 Note that selecting a folder that has been used for output in the past may overwrite existing results. To avoid loss of previous data, select a different folder for each new run.

samtools	<input type="text" value="/Users/xxxx/Desktop/samtools"/>	<input type="button" value="Browse"/>
bcftools	<input type="text" value="/Users/xxxx/Desktop/bcftools"/>	<input type="button" value="Browse"/>

samtools

Select samtools file.

bcftools

Select bcftools file.

Run

1. Enter the path to the file :

fasta	<input type="text" value="/Users/xxxx/Desktop/reference.fa"/>	<input type="button" value="Browse"/>
P1 bam	<input type="text" value="/Users/xxxx/Desktop/P1.sort.bam"/>	<input type="button" value="Browse"/>
P2 bam	<input type="text" value="/Users/xxxx/Desktop/P2.sort.bam"/>	<input type="button" value="Browse"/>
F1 bam	<input type="text" value="/Users/xxxx/Desktop/F1.sort.bam"/>	<input type="button" value="Browse"/>
Abulk bam	<input type="text" value="/Users/xxxx/Desktop/Abulk.sort.bam"/>	<input type="button" value="Browse"/>
Bbulk bam	<input type="text" value="/Users/xxxx/Desktop/Bbulk.sort.bam"/>	<input type="button" value="Browse"/>

fasta

Select fasta file used for alignment.

P1 bam , P2 bam


Select bam files of parents' lineages.

F1 bam

Select the F1 bam file for the parents' lineage.

Abulk bam , Bbulk bam

Select upper or lower bulk bam files

 By default, the path is assumed to be set correctly. If the path is not set or is incorrect, enter it correctly.

Run

2. Enter the name of bam and Enter filtering values

P1 name	P1	P2 name	P2	F1 name	F1	Abulk name	Abulk	Bbulk name	Bbulk
filter depth	10	window size	2000000	step size	50000	bulk number	20		

P1 name

Enter the name of P1.

P2 name

Enter the name of P2.

F1 name

Enter the name of F1.

Abulk name

Enter the name of Abulk.

Bbulk name

Enter the name of Bbulk.

filter depth

Enter the numerical value of the depth to be used for filtering.

window size

Sliding window window size (bp): 2000000

step size

Sliding window step size (bp): 50000

bulk number

Enter the number of bulk

3. Select processing details

fai file	<input checked="" type="radio"/>	Exists	<input type="radio"/>	Not Exists				
Bulk	<input type="radio"/>	single	<input checked="" type="radio"/>	double				
Bulk type	<input type="radio"/>	RIL	<input checked="" type="radio"/>	F2				
pattern	<input type="checkbox"/>	P1	<input type="checkbox"/>	P1, P2	<input checked="" type="checkbox"/>	P1, P2, F1	<input type="checkbox"/>	P1, F1

fai file

Select “Exists” if the fai file is in the same directory as the fasta file, or “Not Exists” if not

Bulk

Select “single” if there is only one Bulk to be analyzed, or “double” if there are two Bulks

Bulk type

Select “RIL” if Bulk is RIL or “F2” if Bulk is F2

pattern

Select the appropriate one according to the bam file used for filtering

⚠ Leave the path and name of the bam file not selected in pattern blank or as is.

Output

QTL-seq_GUI generates three types of folders.

```
<Output folder>
```

```
|---bam
```

```
|---txt
```

```
|---result
```

The files output to each folder are described on the following pages.

bam

Example of output file when the following contents are selected

fai file	<input checked="" type="radio"/>	Exists	<input type="radio"/>	Not Exists				
Bulk	<input type="radio"/>	single	<input checked="" type="radio"/>	double				
Bulk type	<input type="radio"/>	RIL	<input checked="" type="radio"/>	F2				
pattern	<input type="checkbox"/>	P1	<input type="checkbox"/>	P1, P2	<input checked="" type="checkbox"/>	P1, P2, F1	<input type="checkbox"/>	P1, F1

bam

```
|---P1_chrxxx.bam
|---P1_chrxxx.bam.bai
|---P2_chrxxx.bam
|---P2_chrxxx.bam.bai
|---F1_chrxxx.bam
|---F1_chrxxx.bam.bai
|---Abulk_chrxxx.bam
|---Abulk_chrxxx.bam.bai
|---Bbulk_chrxxx.bam
|---Bbulk_chrxxx.bam.bai      etc
```

bam file and bam bai file are output, split by chromosome

txt

Example of output file when the following contents are selected

fai file	<input checked="" type="radio"/>	Exists	<input type="radio"/>	Not Exists				
Bulk	<input type="radio"/>	single	<input checked="" type="radio"/>	double				
Bulk type	<input type="radio"/>	RIL	<input checked="" type="radio"/>	F2				
pattern	<input type="checkbox"/>	P1	<input type="checkbox"/>	P1, P2	<input checked="" type="checkbox"/>	P1, P2, F1	<input type="checkbox"/>	P1, F1

txt

|---bam_file_data_volume.txt

|---snpindex_marge.txt

|---average_snp_index_per_window.txt

|---confidence_interval.txt

|---Abulk-Bbulk_peak.txt

bam_file_data_volume.txt

This file contains information on the amount of data in the bam file divided by chromosomes. bam file name, amount of data, and storage location are described in tab-delimited format.

bam file name	data volume	Location
P1_chr1.bam	419K	/Users/xxxx/Desktop/output/P1_chr1.bam
P2_chr1.bam	419K	/Users/xxxx/Desktop/output/P2_chr1.bam
F1_chr1.bam	415K	/Users/xxxx/Desktop/output/F1_chr1.bam
Abulk_chr1.bam	429K	/Users/xxxx/Desktop/output/Abulk_chr1.bam
Bbulk_chr1.bam	429K	/Users/xxxx/Desktop/output/Bbulk_chr1.bam

If it exceeds 700 Mb, split the bam file by position so that it is less than 500 Mb.

snpindex_marge.txt

This file contains selected SNP information from the vcf file for set values \geq depth , P1, P2 homozygous and F1 heterozygous locations. Chromosome name, position, SNP-index and depth information for each sample are listed in tab-delimited format.

①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪	⑫	⑬
chr1	3500	0	25	1	25	0.556	18	0.333	21	0.483	29	-0.15
chr1	4500	0	19	1	19	0.471	17	0.571	14	0.429	21	0.142
chr2	7500	1	22	0	22	0.52	25	0.368	19	0.565	23	-0.197
chr2	8500	0	19	1	19	0.5	16	0.333	15	0.529	17	-0.196
chr3	10500	0	14	1	14	0.526	19	0.48	25	0.235	17	0.245
chr3	11500	0	17	1	17	0.35	20	0.25	12	0.452	31	-0.202
chr4	12500	0	22	1	22	0.5	18	0.556	18	0.471	17	0.085
chr4	15500	0	17	1	17	0.652	23	0.5	22	0.4	20	0.1

- ① ; chr
- ② ; position
- ③ ; P1 snp-index
- ④ ; P1 depth
- ⑤ ; P2 snp-index
- ⑥ ; P2 depth
- ⑦ ; F1 snp-index
- ⑧ ; F1 depth
- ⑨ ; Abulk snp-index
- ⑩ ; Abulk depth
- ⑪ ; Bbulk snp-index
- ⑫ ; Bbulk depth
- ⑬ ; delta snp-index

average_snp_index_per_window.txt

This file contains information from the snp-index text to the average value of the snp-index for each bulk and delta within the window size. Information on the chromosome name, middle position, start position, end position, and average snp-index for each sample is tab-delimited.

①	②	③	④	⑤	⑥	⑦
chr1	1000000	0	2000000	0.53	0.43	0.096
chr1	1050000	50000	2050000	0.58	0.45	0.125
chr2	1000000	0	2000000	0.77	0.36	0.416
chr2	1050000	50000	2050000	0.76	0.32	0.444
chr3	1000000	0	2000000	0.5	0.43	0.071
chr3	1050000	50000	2050000	0.53	0.39	0.136

① ; chr

② ; middle position

③ ; start position

④ ; end position

⑤ ; Abulk snp-index

⑥ ; Bbulk snp-index

⑦ ; delta snp-index

confidence_interval.txt

This file contains the confidence intervals for the depths. It contains tab-separated values for the chromosome name, intermediate position, start position, end position, and the 99% and 95% confidence intervals for the depths calculated using the simulation file.

①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪	⑫
chr1	1000000	0	2000000	0.15	0.86	0.225	0.78	-0.511	0.52	-0.386	0.396
chr1	1050000	50000	2050000	0.15	0.85	0.226	0.78	-0.507	0.51	-0.379	0.392
chr2	1000000	0	2000000	0.15	0.86	0.228	0.78	-0.51	0.51	-0.389	0.4
chr2	1050000	50000	2050000	0.15	0.87	0.227	0.79	-0.516	0.52	-0.394	0.41
chr3	1000000	0	2000000	0.15	0.86	0.221	0.79	-0.512	0.52	-0.391	0.402
chr3	1050000	50000	2050000	0.14	0.86	0.22	0.79	-0.517	0.53	-0.389	0.402

- ① ; chr
- ② ; middle position
- ③ ; start position
- ④ ; end position
- ⑤ ; Low mean snp-index at 99% confidence interval
- ⑥ ; Higher mean snp-index at 99% confidence interval
- ⑦ ; Lower mean snp-index at 95% confidence interval
- ⑧ ; Higher mean snp-index at 95% confidence interval
- ⑨ ; Low delta mean snp-index at 99% confidence interval
- ⑩ ; High value of delta mean snp-index at 99% confidence interval
- ⑪ ; Low delta mean snp-index at 95% confidence interval
- ⑫ ; High value of delta mean snp-index at 95% confidence interval

Abulk-Bbulk_peak.txt

This file contains the position and SNP-index values for each variety beyond the 95% confidence interval. Chromosome name, start position, end position, and peak position are described in tab-delimited format.

①	②	③	④	⑤	⑥	⑦
chr2	0	2050000	1050000	0.76	0.32	0.444
chr03	0	5000000	1300000	0.866	0.183	0.684
chr03	22200000	25000000	23500000	0.858	0.344	0.515
chr08	0	5100000	1800000	0.877	0.252	0.625

① ; chr

② ; start position

③ ; end position

④ ; peak position

⑤ ; Abulk snp-index

⑥ ; Bbulk snp-index

⑦ ; delta snp-index

result

Example of output file when the following contents are selected

fai file	<input checked="" type="radio"/>	Exists	<input type="radio"/>	Not Exists				
Bulk	<input type="radio"/>	single	<input checked="" type="radio"/>	double				
Bulk type	<input type="radio"/>	RIL	<input checked="" type="radio"/>	F2				
pattern	<input type="checkbox"/>	P1	<input type="checkbox"/>	P1, P2	<input checked="" type="checkbox"/>	P1, P2, F1	<input type="checkbox"/>	P1, F1

result

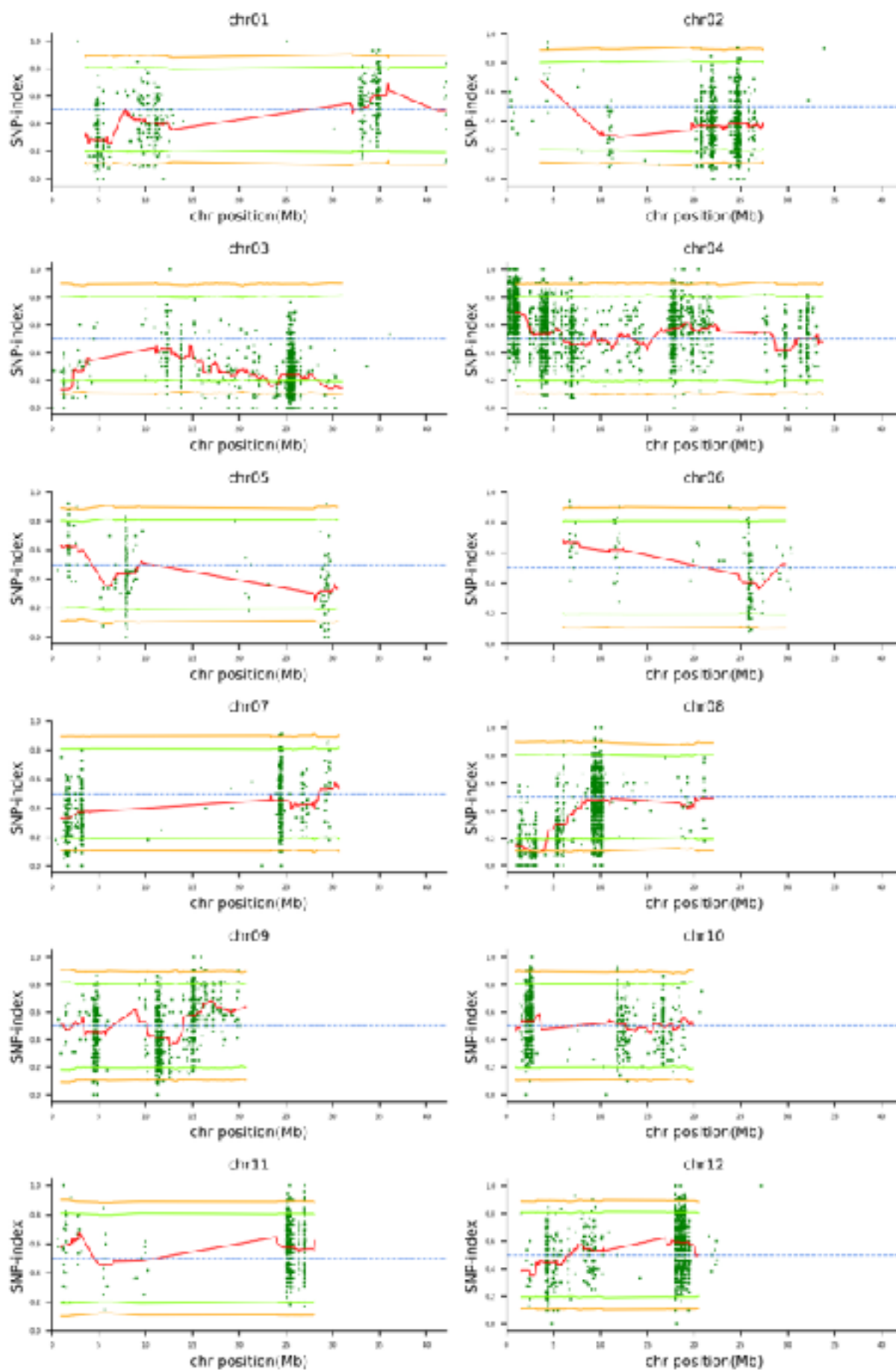
|---Abulk.png

|---Bbulk.png

|---Abulk-Bbulk.png

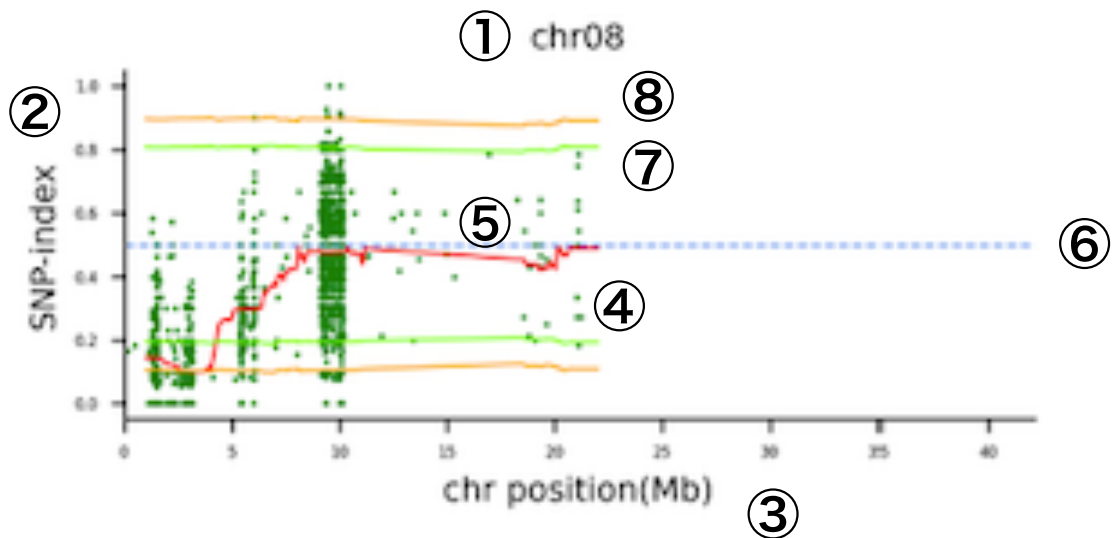
result

Abulk.png



result

Abulk.png



① : Chromosome name

② : SNP-index ($0.0 < \text{SNP-index} < 1.0$)

③ : position (Mb)

④ : SNP

Represents SNPs for position

⑤ : Average SNP-index

Average SNP-index value for Window size

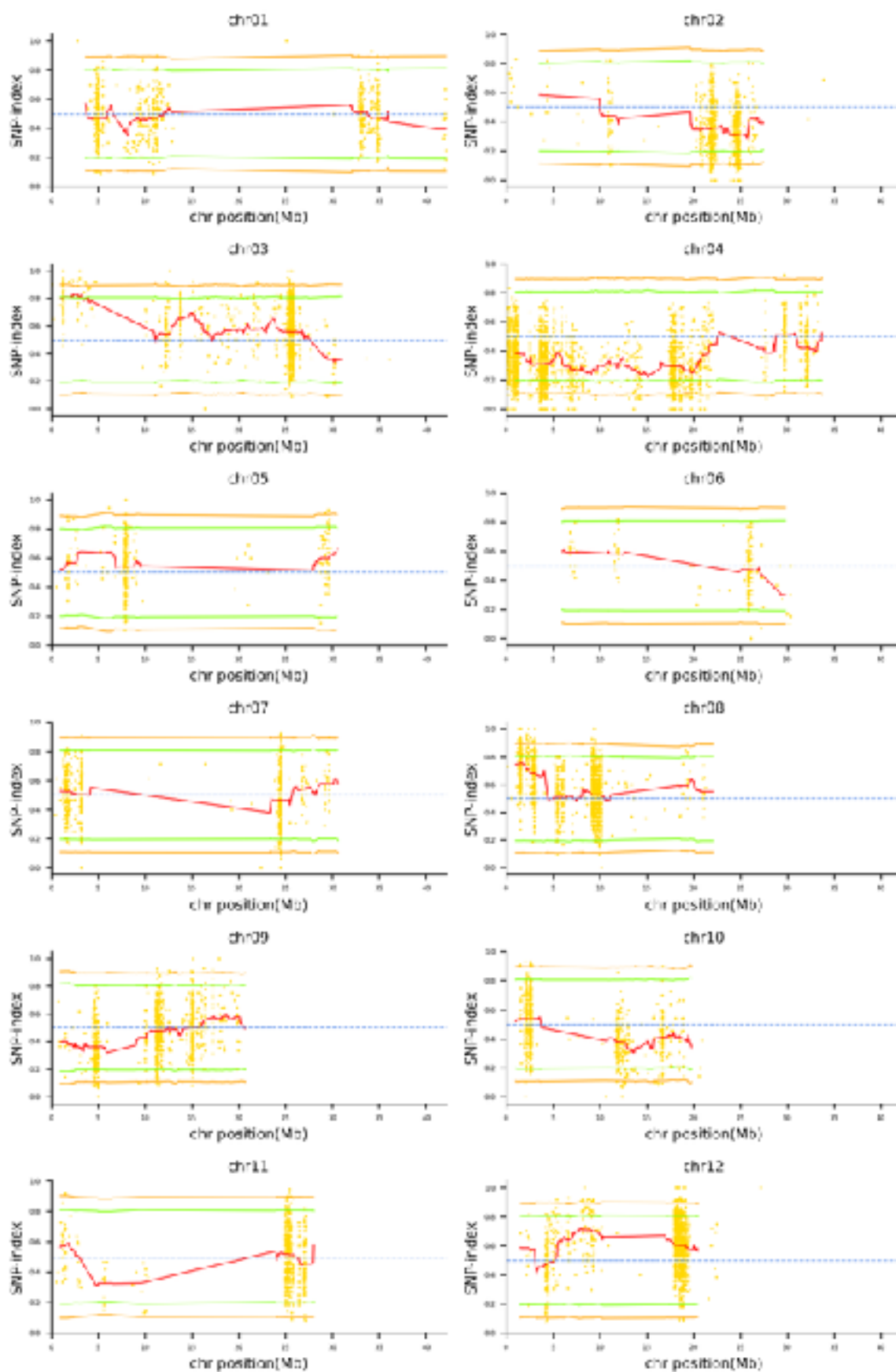
⑥ : SNP-index = 0.5

⑦ : 95% confidence interval for depth

⑧ : 99% confidence interval for depth

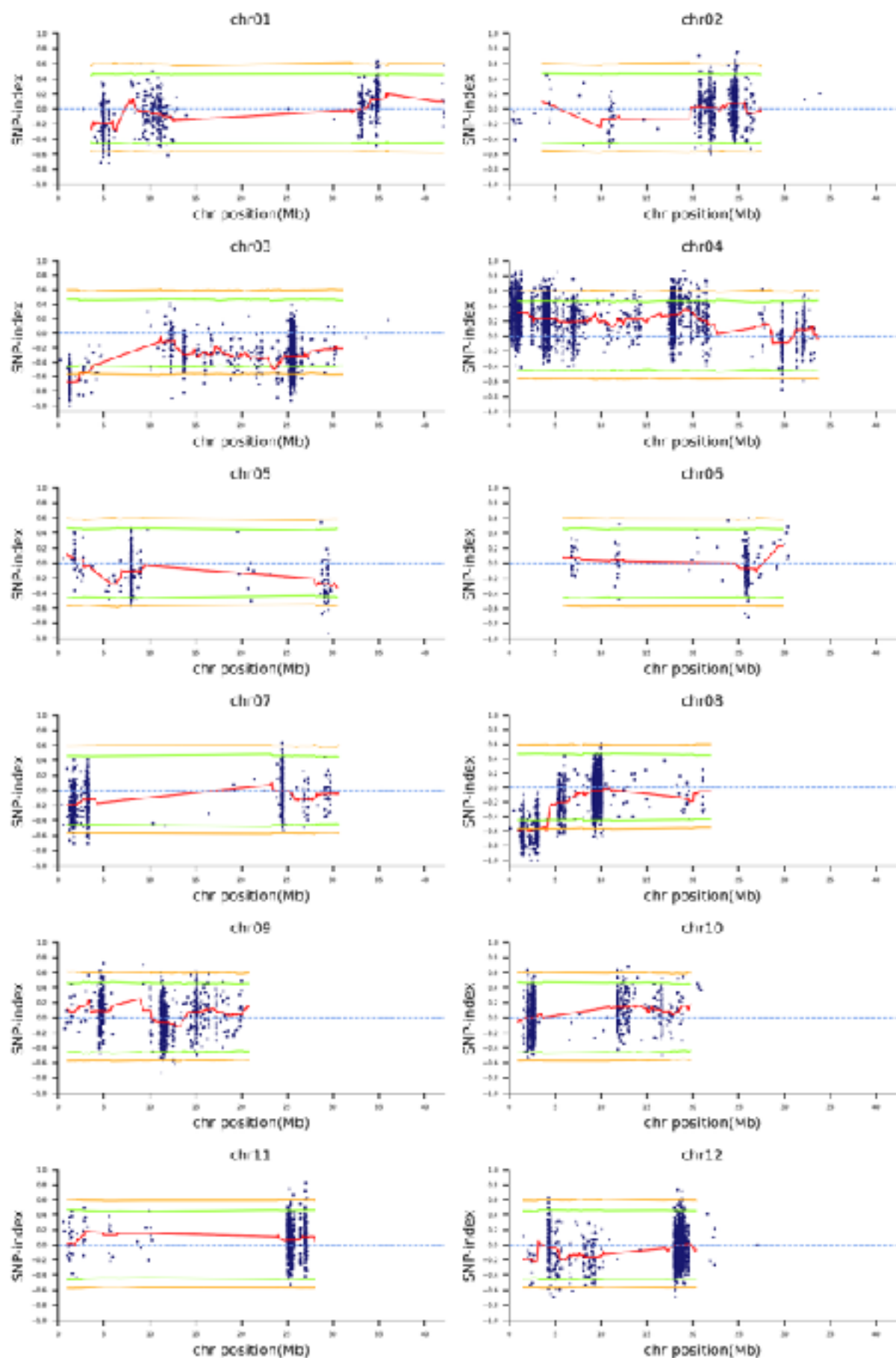
result

Bbulk.png



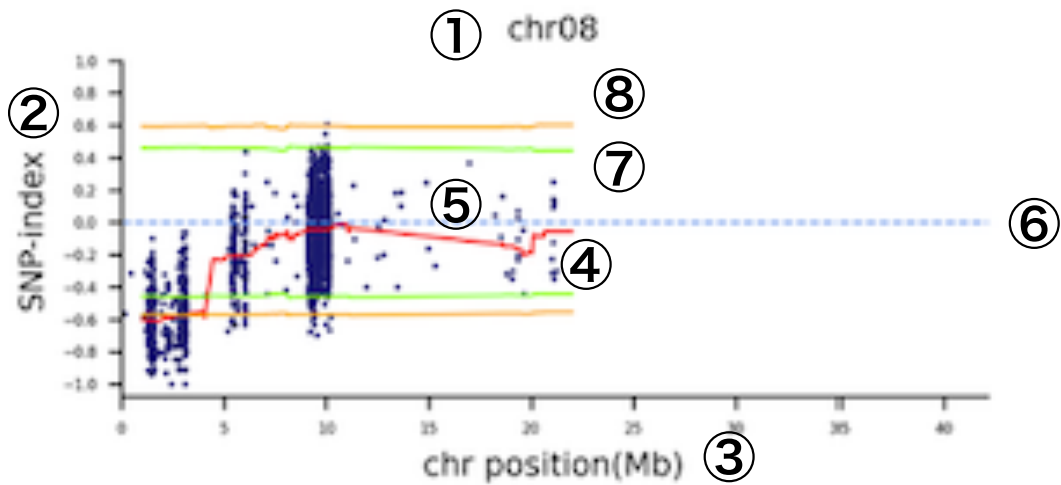
result

Abulk-Bbulk.png



result

Abulk-Bbulk.png



① : **Chromosome name**

② : **SNP-index** ($-1.0 < \text{SNP-index} < 1.0$)

③ : **position (Mb)**

④ : **SNP**

Represents SNPs for position

⑤ : **Average SNP-index**

Average SNP-index value for Window size

⑥ : **SNP-index = 0.0s**

⑦ : **95% confidence interval for depth**

⑧ : **99% confidence interval for depth**