

Short Tutorial

Step 0: Start AntMap



AntMap.exe

Fig. 1

When you use AntMap on Windows, start AntMap with double-clicking the “AntMap” icon (Fig. 1). For other operating systems (i.e., platforms), See below.

Box 1.

Linux and Solaris

Before executing “AntMap-linux” or “AntMap-solaris”, you should change mode of these files to be executable. To do that, type

```
chmod 755 AntMap-xxxx
```

on your command line system (“xxxx” should be “linux” or “solaris”). After changing the mode of files, you can execute AntMap by clicking the “AntMap-linux” or “AntMap-solaris” file icon.

Mac OS X

Note that you can execute “AntMap-macx” from the command line, but cannot execute by clicking the “AntMap-macx” file icon.

AntMap can also be executed by using the executable jar file “AntMap.jar” on any platforms (Linux, Solaris and Mac OS as well as Windows). To execute the jar file, run:

```
java -jar AntMap.jar
```

on your command line system. Some platforms may have bindings already set up such that you can execute the jar file just by clicking on the “AntMap.jar” file icon, which will run the command line equivalent. Note that you should change mode of the jar file to be executable when you are on “Linux” or “Solaris” platforms as described in Box 1.

Step 1: Open an input file.

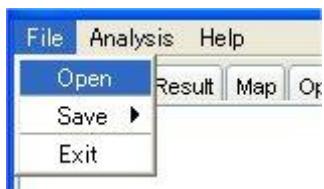


Fig. 2

Open an input file in MapMaker format (*.raw) through “File-Open” menu (Fig. 2). Here, open “sample.raw” contained in the “antmap” folder.

The screenshot shows the Antmap software with the 'Data' tab selected in the top navigation bar. The main panel displays a large amount of raw genetic data in a tabular format. The columns represent individuals (160) and markers (113). The data consists of a series of binary alleles (H or B) separated by spaces. The first few lines of data are:

```
data type f2 intercross
160 113 0

*C1M1      AHBBHAABH-HBBBBHBHAABHHBHBABHHBBH-AHH-BHBAAAHHHHABHHAHHAHABAB
          BAAHBBHHAAAHABAHHAHAHHHBAAHBBB-AHHHHBBHAAHHHHAAAB-HHHAAABABHBB
          AHAHHBAAAHABAABHBAABHBB-AHH-HHBBH-BBB-BBB
*C1M2      AHBBAAABAHBBBBBHBIHABHBBHABHHBBHAAHHABHHAHHHHHHBHHAAABABAB
          B-AHHBAHHAHABHHAHAAHHHHABHAA-HHABHAAHHHHBBHAAHHHHAAABHHHHAAABHBHBB
          AAAHHBAAAHABAABHBAABHBBBAHHHHBHH-BHBBHBBB
*C1M3      HHBBAAABAHBBBBBHBIHABHBBHABH-HBBHAAHHABHHAHHHHHHBHHAAABABAB
          BAAHHBAHHHHAAHAAHHHHABHAAHHHHABAAHHHH-BBHAHHHHAAABHHHHAAHHHHBBH
          A-AHHBAAAHABAABHBBBAABHBBBAHHHHBHHHHBBHBB
*C1M4      HHBBHAAAHBAHHBBHBBH-BHBBHAAH-BBBHAAHHAABHHAHHHHBBHAAAHABAB
          BAAHABAHBHHAAHABHBAHHBAHHHABHAAHHHHBBHAAAHBBHAAHHHHBB
          AAAHHBAAAHABAHHHBAHBHBBBAHHHHBHHHHBB--B-
*C1M5      HHBHHHAHBAHHBBBABIHABHHBBH-HHHBBBHHHHAAHHHHAAHHHHBBAAAHABAB
```

Fig. 3

After opening the file, contents of the file will appear in the “Data” panel (Fig. 3).

The screenshot shows the Antmap software with the 'Log' tab selected in the top navigation bar. The main panel displays a log message indicating the file was opened and verified successfully. The log text is:

```
--open and verify data--
open "C:\$Documents and Settings\$iwata\$My Documents\$Data\$MapData\$SAMPLE.RAW" ... ok
  Generation: F2
  # progenies: 160
  # markers: 113
verify data ... ok
```

Fig. 4

Click the “Log” tab, and you can see a summary of the input data (Fig. 4).

Step 2: Segregation ratio test.

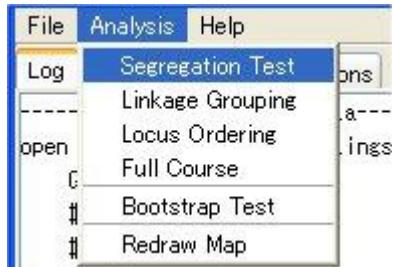


Fig. 5

Select “Segregation Test” from the “Analysis” menu (Fig. 5). Then you can see the results of segregation ratio tests in the “Result” panel (Fig. 6).

A screenshot of the Antmap software interface showing the "Result" panel. The window title is "Antmap". The menu bar includes "File", "Analysis", "Help", "Log", "Data", "Result" (which is highlighted in yellow), "Map", and "Options". The main content area displays a table titled "Segregation Test:" with the following data:

Marker	N	Ratio	Chi^2	P
C1M1	151	46:63:42	4.61	0.0996
C1M2	157	46:69:42	2.51	0.2847
C1M3	157	44:73:40	1.01	0.6028
C1M4	155	42:70:43	1.58	0.4550
C1M5	156	42:78:36	0.55	0.7596
C1M6	157	46:69:42	2.51	0.2847
C1M7	153	50:63:40	6.11	0.0471 *
C1M8	152	49:67:36	4.54	0.1034
C1M9	155	49:71:35	3.66	0.1602
C1M10	153	50:70:33	4.98	0.0831
C1M11	154	43:75:36	0.94	0.6258

Fig. 6

Step 3: Linkage grouping

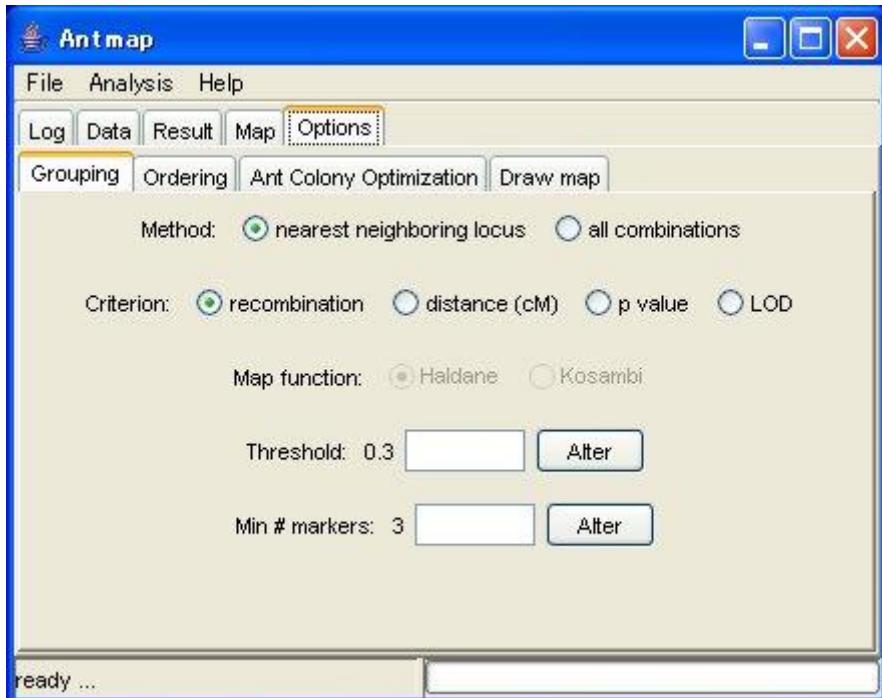


Fig. 7

Click the “Options” tab. Then you can see the “Grouping” option panel (Fig. 7).

You can choose one of the two grouping methods: “nearest neighboring locus” and “all combinations”. The former makes a group by sequentially combining a locus which shows the smallest recombination value against it. This algorithm has been implemented by MAPL (Ukai et al. 1991). The latter will produce similar results with “group” command of MapMaker.

You can also choose the grouping criterion, threshold value and the minimum number of markers for a single group.

Here, we will keep these options unchanged except for the threshold value.

A close-up view of the "Threshold" input field. The text "Threshold: 0.3" is followed by a text input box containing "0.25" and an "Alter" button to its right.

Fig. 8

Type “0.25” into an input area, and push the “Alter” button. Then you can change the threshold value from 0.3 to 0.25.

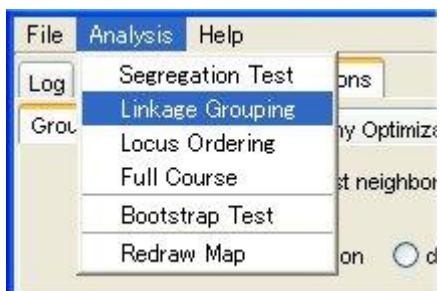


Fig. 9

Select the “Linkage Grouping” from the “Analysis” Menu. Then you can see the results of linkage grouping in the “Result” panel (Fig. 10).

The screenshot shows the "Result" panel of the Antmap software. The panel displays the following text output:

```
Linkage grouping:  
Grouping method: nearest neighboring locus  
Grouping criterion: recombination  
Grouping threshold: 0.25  
Minimum number of markers for each group: 3  
  
No of groups: 6  
Groups # markers  
Group1: 20  
Group2: 20  
Group3: 16  
Group4: 18  
Group5: 20  
Group6: 18  
Group1: 20 markers  
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19  
Group2: 20 markers  
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39  
Group3: 16 markers  
40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55
```

Fig. 10

When you analyze your data, you may not be able to achieve a good separation of markers to linkage groups from the start. In such a case, please find a good set of the threshold value, criterion and method through try-and-errors.

Step 4: Locus ordering

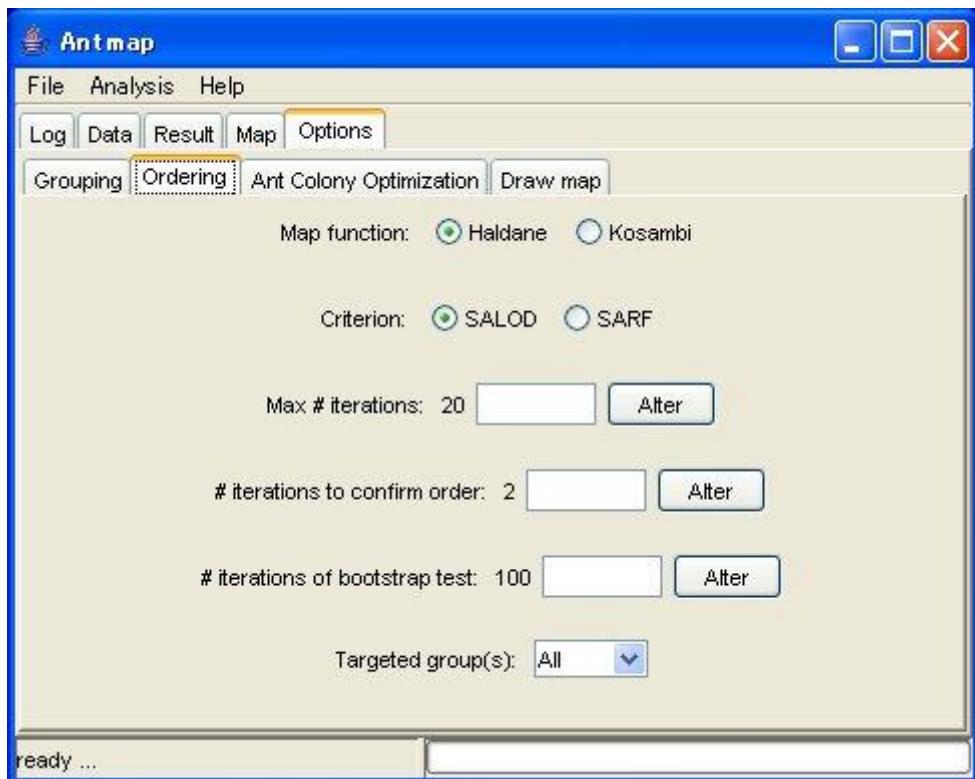


Fig. 11

Click the “Options” tab, and click the “Ordering” tab. Then you can see the “Ordering” option panel (Fig. 11).

In the locus ordering, you can choose one of the two criteria: log-likelihood and “SARF”. “SARF” is an abbreviation for “Sum of Adjacent Recombination Fractions” (Liu 1998). AntMap will search a locus order which maximizes log-likelihood or minimizes “SARF”.

You can also choose the maximum number of iterations and the number of iterations to confirm order. The details of these options are given in the “AntMap Options” section.

A map function for calculating a map distance between adjacent markers can be selected from “Haldane” (Haldane 1919) or “Kosambi” (Kosambi 1944) functions.

Here, we will keep these options unchanged.

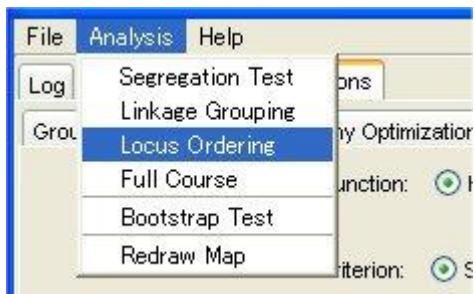


Fig. 12

Select the “Locus Ordering” from the “Analysis” Menu. Then you can see the results of locus ordering in the “Result” panel (Fig. 13).

Antmap		
File	Analysis	Help
Log	Data	Result
Map	Options	
<hr/>		
Group1:		
0	C1M1	0.00
1	C1M2	7.07
2	C1M3	9.77
3	C1M4	18.24
4	C1M5	22.47
5	C1M6	29.32
6	C1M7	40.11
7	C1M8	48.36
8	C1M9	53.10
9	C1M10	55.55

Fig. 13

You can also obtain a graphic of linkage map in the “Map” panel (Fig. 14).

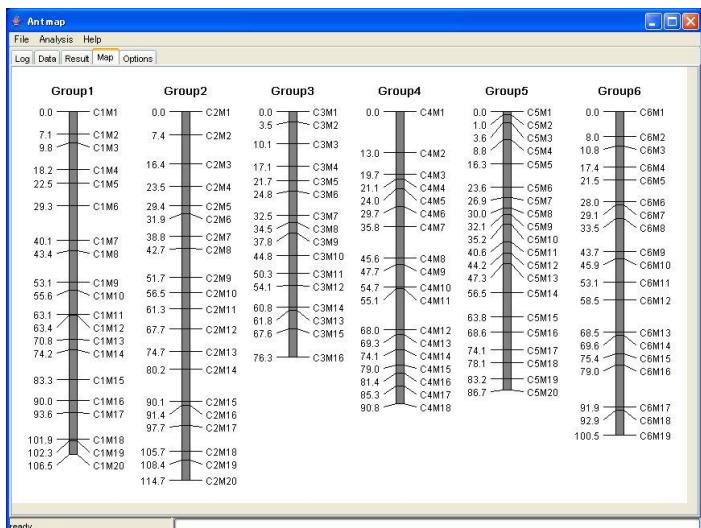


Fig. 14

Step 5: One-step mapping

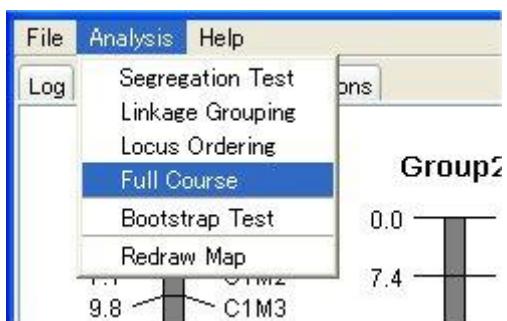


Fig. 15

Select “Full Course” from the “Analysis” Menu. Then, you can overall process from segregation ratio test (Step 2) to locus ordering (Step 4) at once.

Step 6: Redraw a linkage map

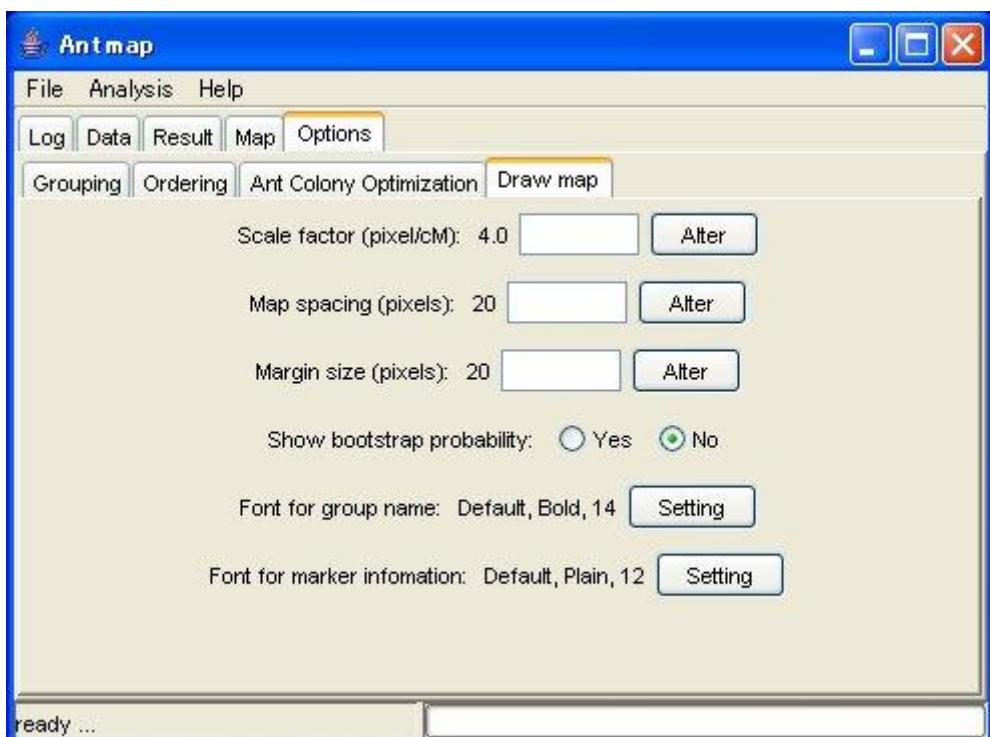


Fig. 16

Click the “Options” tab, and click the “Draw map” tab. Then you can see the “Draw map” option panel (Fig. 16).

Here, we will change the “Scale factor” option. Drawing size of linkage map can be changed through this option. Here, type “2” into an input area, and click the “Alter” button (Fig. 17).

A close-up view of the 'Scale factor (pixel/cM)' input field. The field contains the value '2'. To the right of the field is a button labeled 'Alter'.

Fig. 17

After changing the option value from 4 to 2, select “Redraw Map” from the “Analysis” menu. Then, you can obtain a smaller linkage map than one obtained previously (Fig. 18).

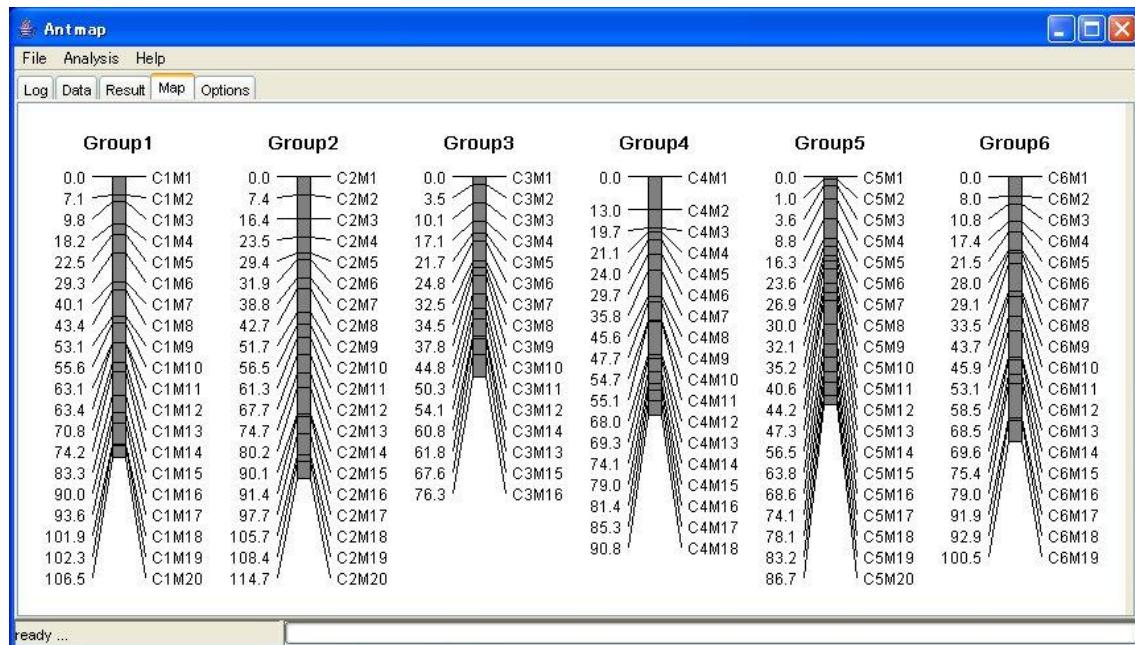


Fig. 18

Step 7: Bootstrap test for locus order

You can evaluate the reliability of estimated locus order by using bootstrap test. Bootstrap test (or bootstrapping) is a method for estimating the sampling distribution of an estimator by resampling with replacement from the original sample. In a bootstrap test, a random sample of size n is drawn from the original sample of size n , and estimates are obtained from the random sample. After repeating (iterating) this operation many times (e.g., 100-1000 times), the stability of estimates (e.g., standard error or confidence interval of estimators) is evaluated. For the details of bootstrap test, please see a good textbook such as Manly (1998). In the bootstrap test for locus order, we can obtain probability that a locus is located at its estimated order (Liu 1998).

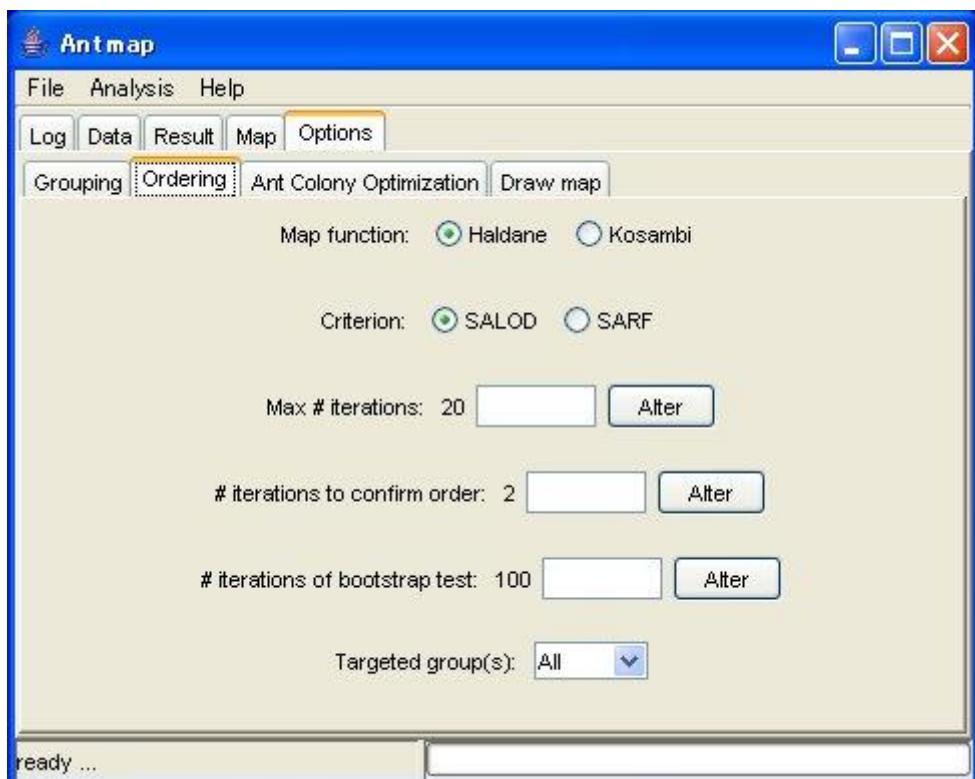


Fig. 19

Click the “Options” tab, and click the “Ordering” tab. Then you can see the “Ordering” option panel (Fig. 19).

You can change the number of iterations (repeats) of bootstrapping. To get a good estimate of percentage of correct locus order, 100 may be sufficient.

You can also choose a group which is targeted in the bootstrap test. Here, we will choose only Group3 to save our time (Fig. 20).

Targeted group(s): Group3

Fig. 20

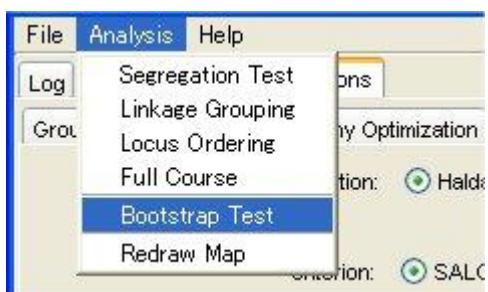


Fig. 21

Select the “Bootstrap Test” from the “Analysis” Menu (Fig. 21). Then you can see the results of bootstrap test for locus order in the “Result” panel (Fig. 22).

Antmap		
File	Analysis	Help
Log	Data	Result

Group3:		
40	C3M1	87.0
41	C3M2	87.0
42	C3M3	100.0
43	C3M4	100.0
44	C3M5	100.0
45	C3M6	100.0
46	C3M7	93.0
47	C3M8	93.0
48	C3M9	100.0
49	C3M10	100.0
50	C3M11	100.0
51	C3M12	100.0
53	C3M14	47.0
52	C3M13	47.0
54	C3M15	100.0
55	C3M16	100.0

Fig. 22

You can also obtain a graphic of linkage map with bootstrap values in the “Map” panel (Fig. 23).

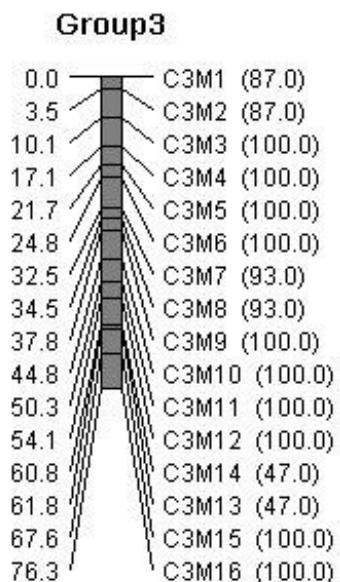


Fig. 23

FYI: The bootstrap test for all linkage groups may take long time even by high-end PC. Thus, you have better set your computer to perform this test at your lunch time or after going home.

Step 8: Save results of linkage mapping

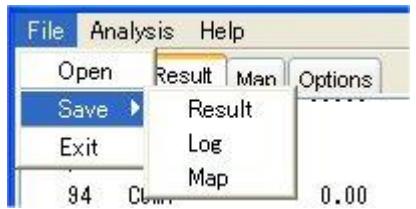


Fig. 24

You can save information in “Result”, “Log” and “Map” panels through the “Save” submenu in the “File” Menu. The information in “Result” and “Log” is saved as a text file. The information in “Map” (i.e., a graphic of linkage map) is saved as a JPEG (*.jpg) file.