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.

File Analy	sis He	lp	
Open	Result	Мар	0
Save 🕨			
Exit			

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.

🍓 Ant	map	
File A	nalysis Help	
Log Da	ata Result Map Options	
data ty	pe f2 intercross	
160 113	0	
*C1M1	АНВВНААВН-НВВЕНВНААВННВНАВНННВВН-АНН-ВНВАААНННАНВННААНАНАВАВ	
1000	ваанввнинаанавааннананнванннавва-ининввнанннааав-иннаававнвн	
	АНААНВААНААВАНВВААВННВ-АНН-ННВНН-ВВВ-ВНВ	
*C1M2	анввааавнанвввнвннавнввнавнннввнааннавннааааннннвннанаававав	
	в-аннваннаннаванннананнва-ннавнаннннввнанннааавнннаавнвнвн	
	АААННВААНААВАНВВААВННВВАНННННВН- ННВВНВВВ	
*C1M3	ННВВАААВНАНВВВНВННАВНВВНАВН-НВВНААННАВННААААННННВННАНААВАВАВ	
	вааннванннннананннананнванннаваанннн-ввнанннааавннннаанннввн	
	А-АННВААНААВННВВААВННВВАНННННВННННВВНВВВ	
*C1M4	ННВВНААНВАННВВНВНН- ВНВВНАНН- ВВВНАННААВННАААНННННВВААНАНВАВАВ	
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	ваанаванвнинананввананиванинавнаниниввааниваааввивнааннивви	
	АААННВААНААВНННВАНВННВВАНННННВННННВВВ-	
*C1M5	ННВНННАНВАННВВАВННАННВВН- НННВВВННННААНННАА	Fig.

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

👙 Antmap	
File Analysis Help	
Log Data Result Map Options	
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	
nereszerezz interesz szterz Aeros	

Fig. 4

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

Step 2: Segregation ratio test.

File	Analysis <mark>Help</mark>	
Log	Segregation Test	ons
	Linkage Grouping	a
open	Locus Ordering	lines
орсп С	Full Course	11185
t	Bootstrap Test	
#	Redraw Map	
	5717 T	ig. −

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).

🆀 Antr	пар				
File An	nalysis He	lp			
Log Da	ta Result	Map Option	ns		
Segregat	ion Test:	5555¢			
Marker	N	Ratio	Chi^2	Р	
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	
C1 M9	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	Fi

Step 3: Linkage grouping

🚔 Antmap 📃 🗖 🔀	
File Analysis Help	
Log Data Result Map Options	
Grouping Ordering Ant Colony Optimization Draw map	
Method: 💿 nearest neighboring locus 🔵 all combinations	
Criterion: ③ recombination 🦳 distance (cM) 🔵 p value 🔵 LOD	
Map function: 💿 Haldane 💿 Kosambi	
Threshold: 0.3	
Min # markers: 3 Alter	
ready	Fic

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.

0.3	0.25	Alter	
	0.3	0.3 0.25	0.3 0.25 Alter

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.

File	Analysis Help	
Log	Segregation Test	ons
Cro	Linkage Grouping	
Grou	Locus Ordering	ny Optimiza
	Full Course	st neighbor
	Bootstrap Test	
	Redraw Map	on 🔿 d
		Fig

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

👙 Antmap 📃
File Analysis Help
Log Data Result Map Options
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
Group): 20
Group2: 20
Group3: 16
Group4: 18
Group5: 20
Group6: 19
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

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

👙 Antmap 📃 🗖 🔀	
File Analysis Help	
Log Data Result Map Options	
Grouping Ordering Ant Colony Optimization Draw map	
Map function: 💿 Haldane 🚫 Kosambi	
Criterion: 💿 SALOD 🚫 SARF	
Max # iterations: 20 Alter	
# iterations to confirm order: 2	
# iterations of bootstrap test: 100	
Targeted group(s): All	
ready	Fia 1

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.

File	Analysis <mark>Help</mark>	
Log	Segregation Test Linkage Grouping	ons
Grou	Locus Ordering	hy Optimization
	Full Course	unction: 💿 H
	Bootstrap Test	
	Redraw Map	

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

12

🌢 Antr	пар			
File An	alysis He	elp		
Log Dat	ta Result	Мар	Options	
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		43.36	
8	C1M9		53.10	
9	C1M10		55.55	

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

ntmap								
e Analysis Help								
a Data Result Map Options								
Group1	Group2	Group3	Group4	Group5	Group6	L		
0.0 C1M1 0 7.1 C1M2 7 9.8 C1M3 7 9.8 C1M3 7 9.8 C1M3 7 9.3 C1M4 16 2.5 C1M5 23 9.3 C1M6 29 10.1 C1M7 38 13.4 C1M8 42 13.1 C1M1 65 15.6 C1M10 55 13.1 C1M10 55 13.1 C1M11 65 13.1 C1M11 7 13.2 C1M11 7 13.2 C1M11 7 14.2 C1M11 7 15.2 C1M11 10 15.2	0.0 C2M1 7.4 C2M2 8.4 C2M3 3.5 C2M4 9.4 C2M6 8.8 C2M6 7.7 C2M8 8.7 C2M10 1.3 C2M10 7.7 C2M12 0.2 C2M12 0.2 C2M13 0.2 C2M14 0.1 C2M15 7.7 C2M13 0.2 C2M14 0.1 C2M16 7.7 C2M13 0.2 C2M14 0.1 C2M16 7.7 C2M18 7.7 C2M14 0.1 C2M17 7.7 C2M18 7.7 C2M17 7.7	0.0 C3M1 3.5 C3M2 10.1 C3M3 17.1 C3M4 21.7 C3M4 21.7 C3M4 22.8 C3M5 22.6 C3M5 34.5 C3M5 34.6 C3M10 54.1 C3M10 54.1 C3M13 54.1 C3M13	0.0 C4M1 13.0 C4M2 13.7 C4M3 21.1 C4M3 21.0 C4M3 23.7 C4M3 23.7 C4M3 23.7 C4M3 24.7 C4M3 54.7 C4M1 68.0 C4M1 6	0.0 C6M1 1.0 C6M2 0.6 C6M3 0.6 C6M1 0.6 C6M2 0.6 C6M1 0.6 C6M2 0.6 C6	0.0 C6M1 8.0 C6M2 10.8 C6M3 17.4 C6M5 28.0 C6M6 29.1 C6M7 33.5 C6M9 45.9 C6M13 53.1 C6M11 55.1 C6M13 56.5 C6M13 56.5 C6M13 57.4 C6M15 79.0 C6M17 29.9 C6M17 29.9 C6M19			
	-							
	L					J		

Step 5: One-step mapping



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

Antmap	
File Analysis Help	
Log Data Result Map Options	
Grouping Ordering Ant Colony Optimization Draw map	-
Scale factor (pixel/cM): 4.0	
Map spacing (pixels): 20 Atter	
Margin size (pixels): 20	
Show bootstrap probability: 🔘 Yes 💿 No	
Font for group name: Default, Bold, 14 Setting	
Font for marker infomation: Default, Plain, 12 Setting	
ady	F

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).

Scale factor (pixel/cM): 4.0	2	Alter	Eia 17
			гı <u>у</u> . т <i>і</i>

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).

Anaiysis Help	ptions				
Group1	Group2	Group3	Group4	Group5	Group6
0.0 C1M1 7.1 C1M2 9.8 C1M3 18.2 C1M3 22.5 C1M4 22.5 C1M6 40.1 C1M7 43.4 C1M6 53.1 C1M7 63.1 C1M10 63.1 C1M11 63.4 C1M10 63.1 C1M11 63.4 C1M12 70.8 C1M13 74.2 C1M13 74.2 C1M13 74.2 C1M13 74.2 C1M15 93.6 C1M17 101.9 C1M18 102.3 C1M19 106.5 C1M20	0.0 C2M1 7.4 C2M2 16.4 C2M3 23.5 C2M4 29.4 C2M5 31.9 C2M6 38.8 C2M7 42.7 C2M8 51.7 C2M9 56.5 C2M10 61.3 C2M11 67.7 C2M13 80.2 C2M14 90.1 C2M14 90.1 C2M14 90.1 C2M15 91.4 C2M18 108.4 C2M19 114.7 C2M20	0.0 3.5 10.1 17.1 24.8 32.5 34.5 37.8 44.8 50.3 50.5 50	0.0 C4M1 13.0 C4M2 19.7 C4M3 21.1 C4M4 24.0 C4M5 29.7 C4M6 29.7 C4M6 35.8 C4M6 C4M7 C4M8 C4M7 C4M10 C4M11 68.0 C4M11 C4M12 69.3 C4M14 C4M12 C4M13 C4M14 C4M15 S1.4 C4M16 S1.4 C4M16 S1.4 C4M17 90.8 C4M18	0.0 1.0 3.6 2.5M3 8.8 16.3 23.6 26.9 30.0 40.6 44.2 47.3 56.5 68.6 74.1 78.1 8.32 2.5M1 C5M3 C5M4 C5M5 C5M6 C5M7 C5M9 C5M10 C5M10 C5M11 C5M12 C5M10 C5M10 C5M11 C5M12 C5M10 C5M10 C5M10 C5M11 C5M12 C5M13 C5M13 C5M14 C5M10 C5M11 C5M11 C5M11 C5M11 C5M12 C5M11 C5M12 C5M2 C	0.0 8.0 10.8 17.4 21.5 28.0 29.1 33.5 53.1 55.1 55.1 56.5 56.6 75.4 79.0 92.9 55.1 56.5 56.6 75.4 79.0 56.1 56

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).

🚔 Antmap 📃 🗖 🔀
File Analysis Help
Log Data Result Map Options
Grouping Ordering Ant Colony Optimization Draw map
Map function: 💿 Haldane 🚫 Kosambi
Criterion: 💿 SALOD 🚫 SARF
Max # iterations: 20 Alter
iterations to confirm order: 2
iterations of bootstrap test: 100
Targeted group(s): All
ready

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).

				Fig.	20
File An	alysis	Help			
Log Grou	Segreg Linkag Locus Full Co	ation Test e Grouping Ordering purse	ons hy Op tion:	timization	
	Bootst Redrav	rap Test v Map	ion:	() SAL (

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	Anal	ysis He	elp				
Log	Data	Result	Map	Options			
Group	3:						
4	40 C	3M1		87.0			
4	41 C	3M2		87.0			
4	42 C	3 M 3		100.0			
4	43 C	3M4		100.0			
4	14 C	3M5		100.0			
4	45 C	3M6		100.0			
4	46 C	3M7		93.0			
4	17 C	3M8		93.0			
4	48 C	3 M9		100.0			
4	49 C	3M10		100.0			
5	i0 C	3M11		100.0			
5	i1 C	3M12		100.0			
5	i3 C	3M14		47.0			
5	i2 C	3M13		47.0			
5	i4 C	3M15		100.0			
5	5 C	3M16		100.0			

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

Group3

0.0	— C3M1 (87.0)	
3.5	└C3M2 (87.0)	
10.1	C3M3 (100.0)	
17.1	C3M4 (100.0)	
21.7	\ C3M5 (100.0)	
24.8 ///	\ [\] C3M6 (100.0)	
32.5 ///	\\C3M7 (93.0)	
34.5 ////	// C3M8 (93.0)	
37.8 ///	∭ C3M9 (100.0)	
44.8 ///	∭ C3M10 (100.0)	
50.3 🕼 🐧	∰ C3M11 (100.0)	
54.1 佣 \	C3M12 (100.0)	
60.8 🎢 🔡	₩C3M14 (47.0)	
61.8 🖞 👘	∦C3M13 (47.0)	
67.6 ⁴	C3M15 (100.0)	
76.3 [/]	¹ C3M16 (100.0)	Eig 22
		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

File Anal	ysis Help		
Open	Result Man	Options	
Save)	Result		
Exit	Log		
94 0	Map	0.00	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.