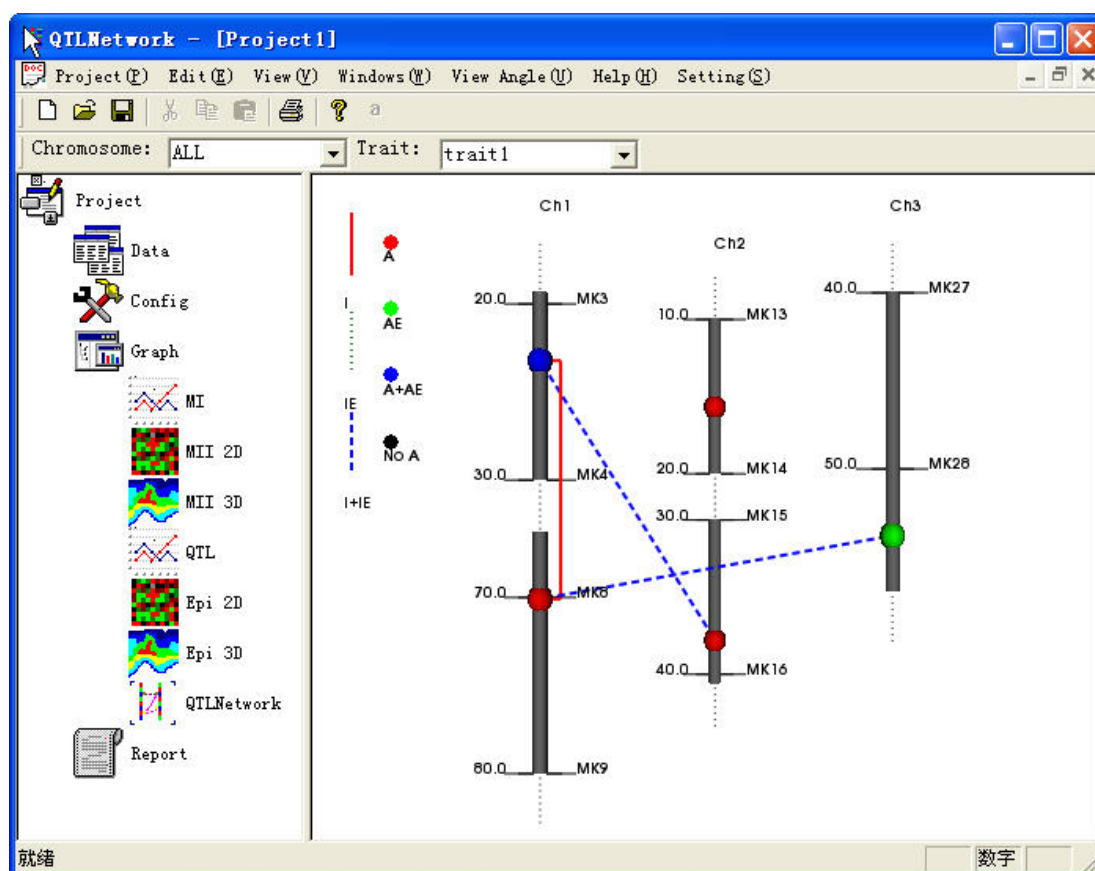


QTLNetwork-2.2 User Manual

Software for Mapping QTL with Epistatic and QE Interaction Effects

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Zhejiang University, China

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Contents

QTLNetwork-2.2 User Manual

1. What's new function in QTLNetwork version 2.2?	4
1.1 Multiple trait joint analysis for QTL mapping	4
1.2 Triplet interacting QTLs detection by 3 dimension genomic scan.....	4
1.3 Individual genetic effects estimation.....	4
1.4 Replication treatment	5
2. Introduction to QTLNetwork	5
3. Installing QTLNetwork	6
4. Running QTLNetwork	9
5. Starting with QTLNetwork	9
5.1 Data Format.....	9
5.1.1 Format of marker linkage map file.....	9
5.1.2 Format of data file	11
5.1.3 Other acceptable data formats	15
5.2 Create new project with map and data files	15
5.2.1 Import source data files	15
5.2.2 Export source data files	16
5.2.3 Start computation	16
5.3 Open the project with map, data and result files	23
6. Interactive Visualization operations	24
7. Config setting in the mapping computation	26
7.1 General page.....	26
7.2 Genome scan configuration page	27
7.3 Significance level configuration page	28

7.4 Output configuration page..... 28

8. Graphic configuration 28

9. Save pictures and reports..... 30

9.1 Save pictures 30

9.2 Save and understand the text reports..... 30

1. What's new function in QTLNetwork version 2.2?

1.1 Multiple trait joint analysis for QTL mapping

In this program, multiple traits value within different environment and different replication could be jointly analysed to detect pleiotropic effects. Please find the keyword “_TraitNumber” in the top of the data file, type the number of traits, and then type index of traits which you want to analyse jointly, with connector “+”, the program will search for pleiotropic QTLs in multiple traits. For instance, there are 4 traits in total, the first two are supposed to be analysed jointly, and so do the last two.

```
_Population      DH
_Genotypes       99
_Observations    198
_Environments     yes
_Replications     yes
_TraitNumber      4 1+2 3+4
_TotalMarker     54
_MarkerCode      P1=1    P2=2    F1=3    F1P1=4  F1P2=5
```

1.2 Triplet interacting QTLs detection by 3 dimension genomic scan

This program is also able to detect possible QTLs involved in triplet interaction. Please find the keyword “_Population” followed with population name. As shown in the following figure, see “T 3D” after population name, where “T” stands for triplet interaction analysis and 3D stands for partial 3D genomic scan, the program would perform triplet QTLs interaction analysis by 3D genomic scan. Also, you can type only the letter “T” to carry out triplet interaction loci detection among individual and epistatic interacting QTLs. Currently, the triplet interaction analysis only works for DH and RIL population.

```
_Population      DH T 3D
_Genotypes       99
_Observations    198
_Environments     yes
_Replications     yes
_TraitNumber      4
_TotalMarker     54
_MarkerCode      P1=1    P2=2    F1=3    F1P1=4  F1P2=5
```

1.3 Individual genetic effects estimation

As plant and animal breeders also hope to utilize the mapping population to produce new variety or inbred lines, by selecting valuable and beneficial individuals. The program is available to estimate individual genetic effects based on estimation effects of QTLs and QTL by

environments interaction effects' prediction.

Please find the keyword “_Genotypes” with the genotype number following. Typing one decimal fraction less than 1 after the genotype number the program would perform individual genetic effects estimation, and present the result in output file (*.qnk). The scale of the presented results is determined by decimal fraction you typed.

Please see also part 11 in section 8.2 for the results of individual genetic effects estimation.

```

_Population      DH
_Genotypes       99 0.1
_Observations    198
_Environments     yes
_Replications     yes
_TraitNumber      4
_TotalMarker     54
_MarkerCode      P1=1    P2=2    F1=3    F1P1=4  F1P2=5

```

1.4 Replication treatment

This program can consider replication either as simple repetition or as block in experiments, that is providing choices that mapping QTLs based on the mean phenotype value or not. Please find the keyword “_Replications” in the figure below, in the case of replication is employed in the experiment, that is, the keyword is followed with “yes”, an additional ‘B’ or ‘b’ after “yes” would let the program treat replications as block in computation, otherwise, the program would perform QTLs mapping based on averaged phenotype value, which means the replications is treated as repetition of experiments.

```

_Population      DH
_Genotypes       99
_Observations    198
_Environments     yes
_Replications     yes B
_TraitNumber      4
_TotalMarker     54
_MarkerCode      P1=1    P2=2    F1=3    F1P1=4  F1P2=5


```

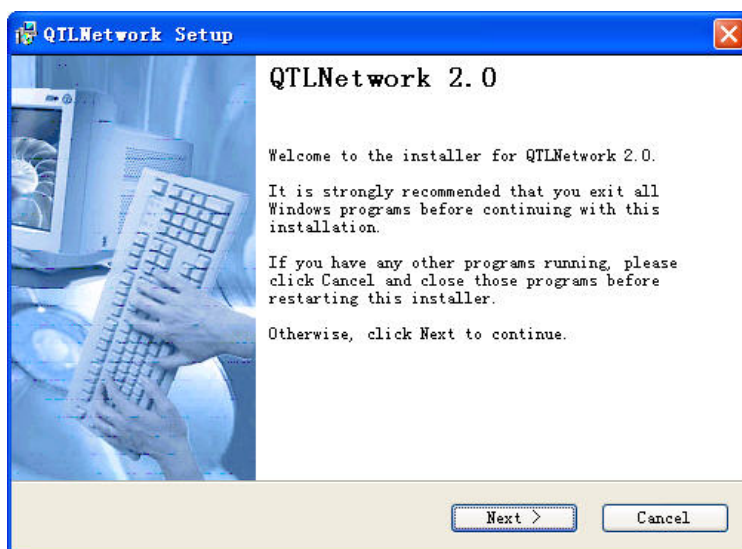
2. Introduction to QTLNetwork

QTLNetwork-2.0 is user-friendly computer software for mapping quantitative trait loci (QTL) with epistatic effects and QTL by environment (QE) interaction effects in DH, RI, BC1, BC2, F2, IF2 and BxFy populations, and for graphical presentation of QTL mapping results. The software is developed based on the MCIM (mixed-model based composite interval mapping) method, and programmed by C++ programming language under Microsoft Visual C++ 6.0 environment. The GUI (Graphic User Interface) of QTLNetwork is developed by MFC (Microsoft Foundation Class) and the graphic visualization is done by VTK (Visualization

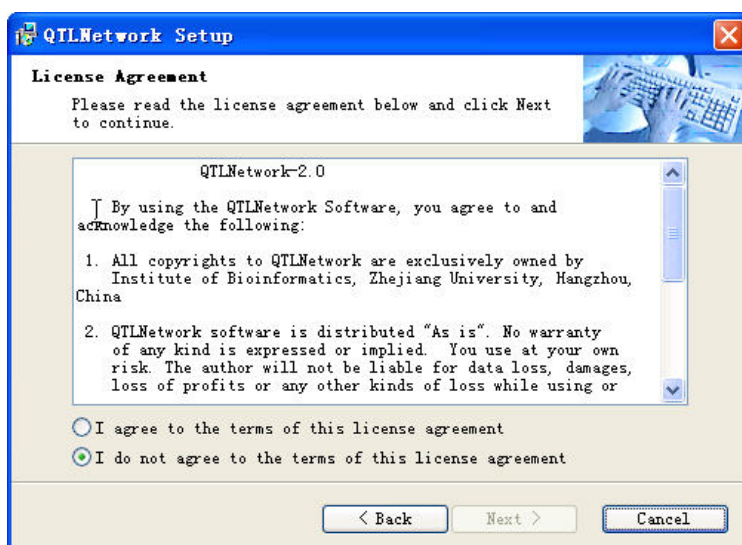
Toolkit). This software works with Microsoft Windows operating systems, including Windows 95/98, NT, 2000, XP, 2003server. A new version of QTLNetwork is under developing, and its functions will be extended to include marker-assisted virtual breeding. Considering that most users are using Microsoft Windows system, we will only describe the application of GUI version of QTLNetwork in the following section.

3. Installing QTLNetwork

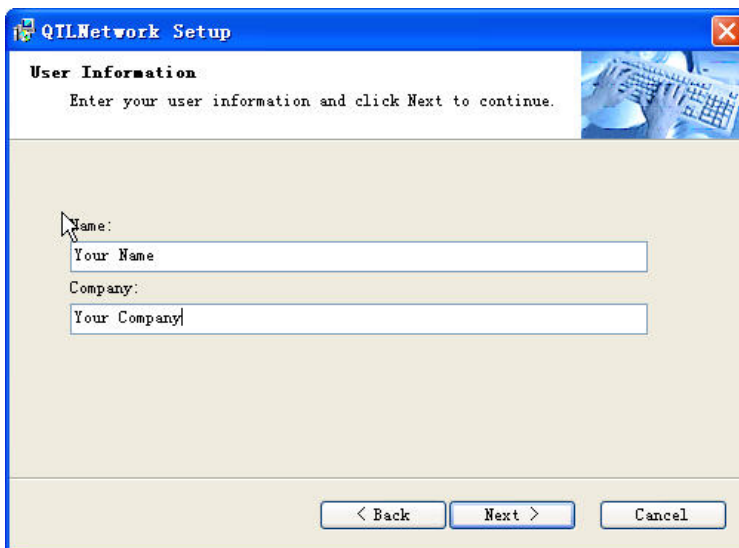
The software is freely available from the URL <http://ibi.zju.edu.cn/software/qtlnetwork/>. Download the QTLNetwork setup package QTLNetwork-2.0-Setup.exe , and double click it. The setup welcome screen displays as following.



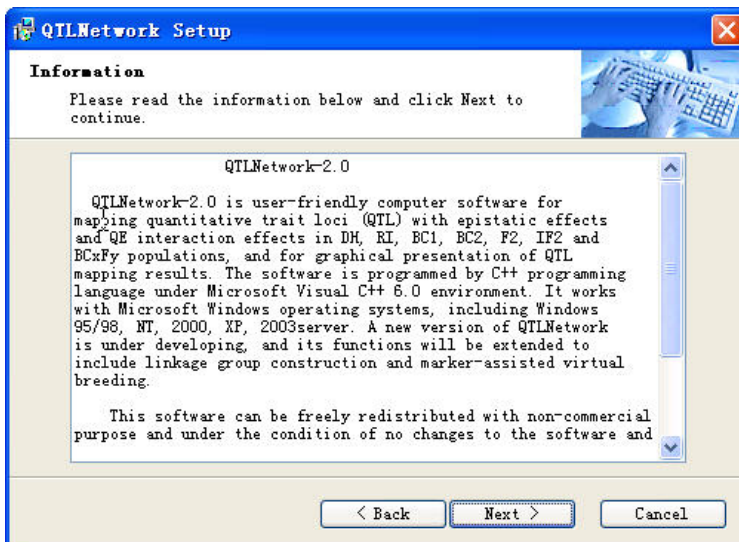
Click the button of next, and enter to the screen of License agreement.



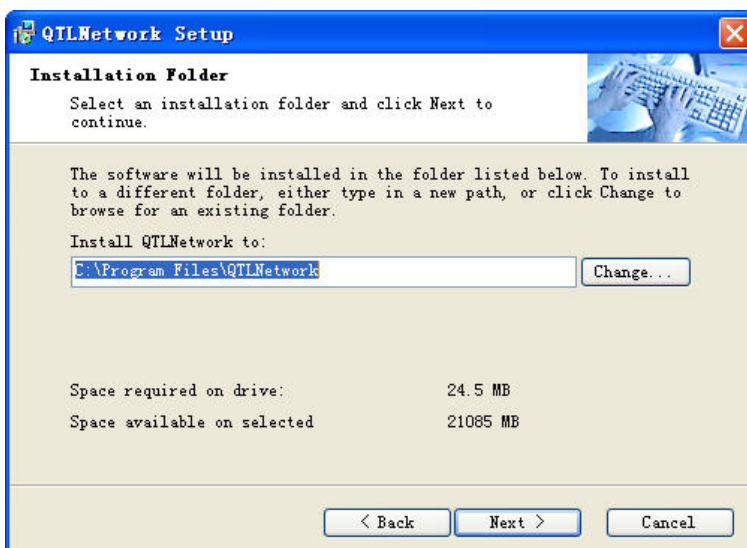
Read the license, and click the radio button of agreement to enter to User Info Screen.



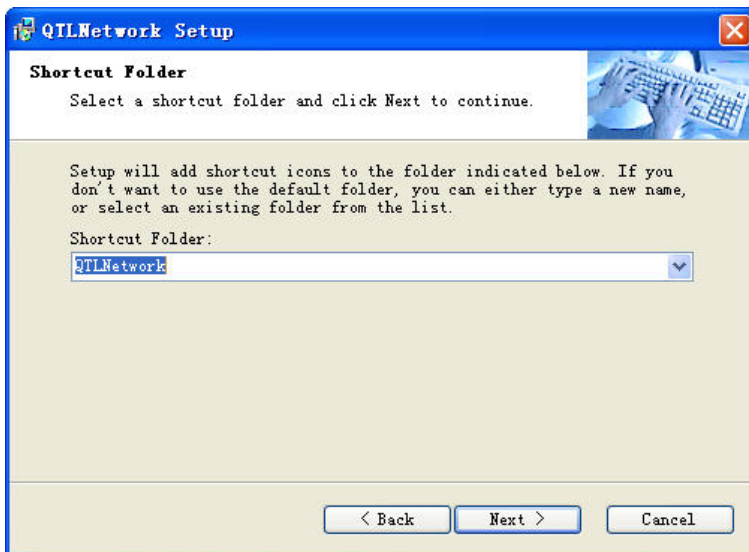
Type names of you and your company, then click the next button to enter to Software Info Screen.



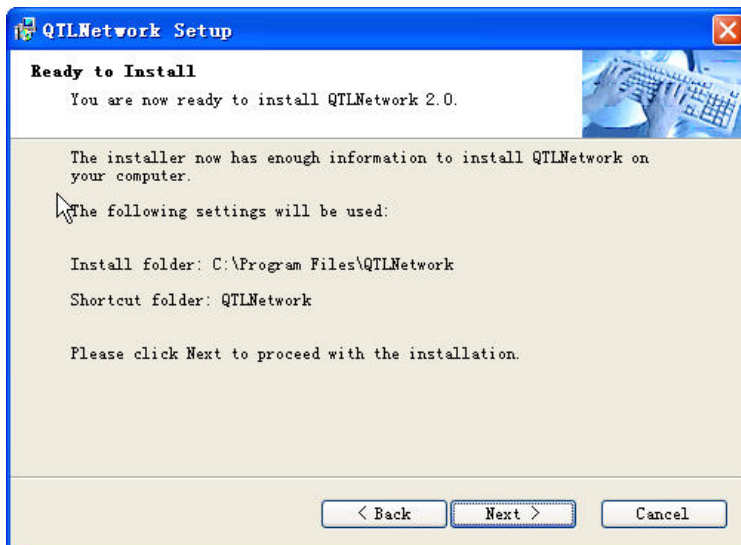
Read the information, and then click the next button to choose the installation folder.



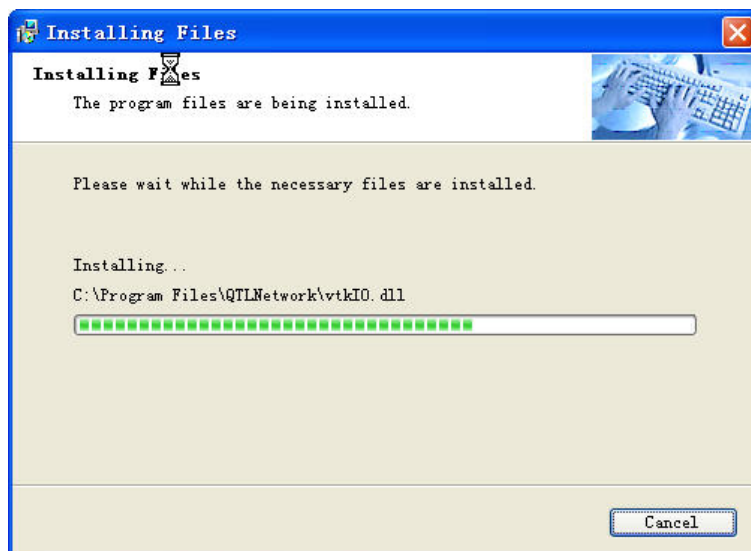
Click the button of “Change...” to browse file directories to install to your purpose folder. Click the next button to the Shortcut Folder Screen.



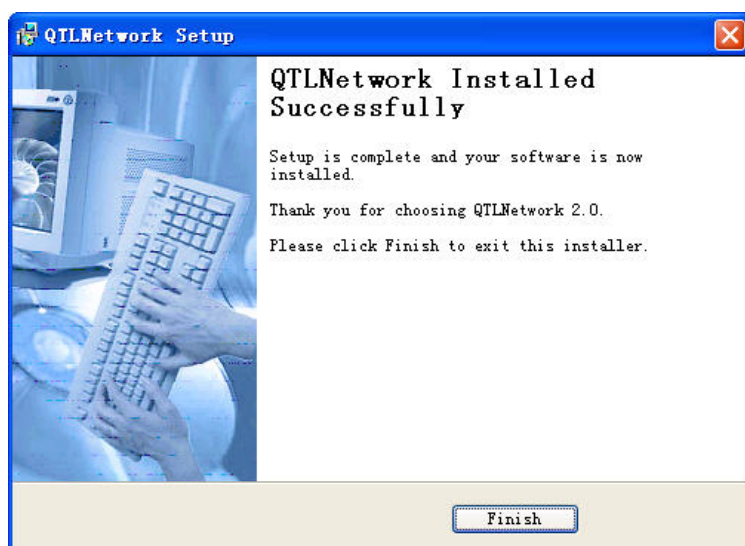
Choose your purpose Shortcut Folder here, and then click the next button to the screen of final check.



Please check the setup information, and then click the next button to start the installation.



Wait till the installation procedure finish.



Press the button of Finish, and the installation is successfully complete.

4. Running QTLNetwork

After successfully install QTLNetwork, users can start to run it in two ways:

Click Start-> Programs ->QTLNetwork ->QTLNetwork-2.0.exe



or click the shortcut  in desktop to run QTLNetwork software.

5. Starting with QTLNetwork

5.1 Data Format

For performing analyses with QTLNetwork 2.0, two source data files are required: a marker linkage map file (for simplification, we call it map file) and a data file. A map file contains information about the order and genetic distances of all observed markers on the chromosomes or linkage groups. A data file contains observations of the markers and the traits under study for all individuals. We provide some sample files for briefly demonstrating the format of source data files for QTLNetwork 2.0 in the sub-directory (\SampleData) where QTLNetwork has been installed. The map and data files for QTLMapper software can be directly used by QTLNetwork 2.0.

5.1.1 Format of marker linkage map file

This file contains information about the marker linkage map, such as the number of

chromosomes, number and order of markers on each of the chromosomes, flanking marker distances, etc. It consists of general description and map body.

General Description: This part is in the front of map file. A typical general description looks like:

```
_DistanceUnit  Cm
_MapFunction  K
_Chromosomes  4
_MarkerNumbers 6 4 7 9
```

There are a total of four possible items for general description. They can be in any order. Each item in general description is a key word followed by certain specification(s). Each key string must be started with an underline “_”, and there should not be any list separator (white space or tab) within the key string. The specification(s) must be separated from the key word by at least one list separator, and there must also be at least one list separator between any two neighboring specifications if two or more specifications are included for the item. A key string and its specification(s) must be placed in the same line. Both key strings and specification(s) (if characters) are not case insensitive.

_DistanceUnit specifies the unit of genetic distances used in the map file. The specification string “cM” stands for centi-Morgan and “M” stands for Morgan.

_MapFunction indicates the map function used in creating the marker linkage map for transforming recombination fractions into genetic distances. Specification character “K” is for Kosambi function and “H” for Haldane function.

_Chromosomes is for specifying the total number of chromosomes or linkage groups involved in the map file.

_MarkerNumbers is for specifying the number of markers on each of the chromosomes. The order of the numbers must be consistent with that for genetic-distance columns in the map body.

Map Body: This part starts from key string *MapBegin* and ends at key string *MapEnd*. A typical map body looks like:

```
*MapBegin*
Marker# Ch1   Ch2   Ch3   Ch4
1   0.00   0.00   0.00   0.00
2   9.84   11.26   7.45   9.85
3  10.22   8.69    9.10  10.93
4   8.25   9.87   10.66  10.70
5   9.79           10.16  10.10
6   7.47           8.34   11.30
7                   11.21   9.30
```

8	7.23
9	11.78
MapEnd	

The strings (Marker#, Ch1, Ch2, Ch3, Ch4) in the second row show the contents of the columns below them. The Marker# column (first column) is for the order of all markers on each chromosome; the maximum order is equal to the number of markers on the chromosome that has the most markers among all the chromosomes. The Ch1 column (second column) to Ch4 column (last column) each represents a chromosome or linkage group, and contains genetic distances between adjacent markers on the chromosome. Specifically, the genetic distance for the first marker on each chromosome must be set to zero as the start point of the linkage map for the chromosome; the distance for the second marker is between the first and the second markers; the distance for the third marker is between the second and the third markers, and so on. The order of Ch1 column (second column) to Ch4 (last column) must be consistent with that for the numbers following the key string _MarkerNumbers.

5.1.2 Format of data file

The data file contains information on population type, number of genotypes sampled from the population, number of observations, observations for both markers and quantitative traits, etc. It is composed of four parts: general description, marker data body, trait data body, and some comment lines.

General description: This part is for specifying the basic features of the data file, and is usually put in the front of the data file. Like in the map file, each item in general description is a key character string followed by certain specification(s). Each key string must be started with an underline “_”, and no white space is allowed within it. There are eight possible items for general description. They can be arranged in any order. A typical description for a data file looks like:

```
_Population DH
_Genotypes 200
_Observations 400
_Environments yes
_Replications no
_TraitNumber 1
_TotalMarker 64
_MarkerCode P1=1 P2=2 F1=3 F1P1=4 F1P2=5
```

_Population specifies the population type used. Some commonly used populations are listed as follows:

RI population – derived from a cross between two pure-line parents. The specification word for RI population can be RI or RIL.

BC population – derived from crossing F1 with one of the inbred parents. The specification

words for BC1 and BC2 populations are B1 and B2, respectively.

F2 population – derived from selfing or sib-mating F1 that is made by crossing two inbred lines.

Immortalized F2 (IF2) population – derived from randomly mating among individuals from DH or RI population (See Ref: Hua JP, Xing YZ, Xu CG, Sun XL, Yu SB and Zhang QF (2002) Genetic dissection of an elite rice hybrid revealed that heterozygotes are not always advantageous for performance. Genetics 162: 1885–1895). The specification word IF2DH is for IF2 population derived from DH population, and IF2RI for that from RI population.

BxFy Population – derived from F1 backcrossing to one of the inbred parents or selfing for several generations. In each generation, selfing, backcrossing or creating double-haploid is permitted. Let take the following designs for instances:

$P1 \times P2$	$P1 \times P2$	$P1 \times P2$	$P1 \times P2$
$F1$	$P1 \times F1$	$F1 \times P2$	$F1 \times P2$
\otimes			
$F2$	$P1 \times B1$	$P1 \times B2$	$P1 \times B2$
\otimes			
$F3$	$P1 \times B1B1$	$B2B1$	$B2B1$
\otimes		\otimes	double-haploid
$F4$	$B1B1B1$	$B2B1F$	$B2B1D$

The specification words for the four designs above are FFF, B1B1B1, B2B1F and B2B1D, respectively.

_Genotypes specifies the total number of genotypes sampled from the mapping population.

_Observations specifies the total number of observations for each trait studied. **_Environments** specifies the status of experimental design for environments. If the experiment is conducted in multiple environments, write the specification word yes after the key word **_Environments**, otherwise write no.

_Replications specifies the status of experimental design for replications or blocks. If the experiment is conducted with replications or blocks, write the specification word yes after the keyword **_Replications**, otherwise write no.

_TraitNumber specifies the total number of traits included in the data file.

_TotalMarker specifies the total number of the markers included in the data file. This number must be equal to the summation of the numbers for **_MarkerNumbers** in the map file.

_MarkerCode defines a marker coding scheme. There are five possible strings for the specifications. Each of the strings looks like an equation, but no white space is allowed within the string. On the left side of the equation symbol is the marker phenotype specification:

P1: Marker phenotype being the same as that of P1;

P2: Marker phenotype being the same as that of P2;

F1: Marker phenotype being the same as that of F1;

F1P1: Marker phenotype that is not P2 type (P1 dominant or undistinguishable between P1 type and F1 type);

F1P2: Marker phenotype that is not P1 type (P2 dominant or undistinguishable between P2 type and F1 type).

On the right side of the equation symbol is the code for the marker type. The marker code should always be a single character (a number or a letter). The symbol dot “.” is used to represent missing marker data or trait value. It is not necessary to specify codes for all possible marker types except for F2 population. For example, if your marker data were collected from a DH population, only the specifications for P1 and P2 types are enough.

Marker data body: This part is embraced by two key strings *MarkerBegin* and *MarkerEnd*. The order of the marker data for different marker loci must be consistent with the order of markers on each chromosome determined in the map file. Since electronic table software usually has a limit on the number of columns in spreadsheet, we provide two types of arrangements for marker data.

Type I:

MarkerBegin

#Ind	Mk1	Mk2	Mk3	Mk4	Mk5	Mk6	Mk7	Mk8	Mk9;
1	1	1	2	2	2	2	1	1	;
2	1	1	.	1	1	2	2	2	2
3	2	.	2	1	1	1	1	2	2
.....									
89	2	2	2	2	.	1	1	.	1
90	1	1	2	2	2	2	2	1	1

MarkerEnd

Type II:

MarkerBegin

#Mk	1	2	3	4	5	...	48	49	50	88	89	90	;
Mk1	1	1	1	2	1	...	2	2	1	...	1	2	1	;
Mk2	1	1	1	.	2	...	1	2	1	...	1	2	1	;
Mk3	1	.	1	2	2	...	1	2	2	...	1	2	2	;
Mk4	2	1	1	1	1	...	1	2	2	...	1	2	2	;
Mk5	2	1	.	1	1	...	1	1	1	...	2	.	2	;
Mk6	2	2	2	1	1	...	1	1	1	...	2	1	2	;
Mk7	2	2	2	1	1	...	2	1	1	...	2	1	1	;
Mk8	2	2	2	2	2	...	2	1	2	...	1	.	1	;

```
Mk9   1  2  2  2  2  ... 2  2  2  ... 2  1  1  ;
*MarkerEnd*
```

The two types of marker data arrangement are distinguished by a keyword placed at the beginning of the send row, the keyword #Ind for type I and #Mk for type II. The marker names and marker data must be arranged in the order given in the map file. Any list separator is not allowed within the marker names. Each row must end with a semicolon “;”.

Trait data body: This part is between two key strings *TraitBegin* and *TraitEnd*. The input of trait data is source-based. The Source includes the environment (if available), the replication (if available) and the genotype, from which the observations was obtained for all the traits studied. The following is an example for the trait data body.

```
*TraitBegin*
Env#   Rep#   Geno#  Trait_1 Trait_2 Trait_3 ;
1  1  1  2.44   7.40   10.04  ;
1  1  2  2.40   4.32   8.55   ;
.....
1  1  90 3.54   8.19   10.74  ;
1  2  1  3.17   6.91   11.86  ;
1  2  2  1.90   4.31   11.36  ;
.....
1  2  90 3.22   10.54  11.48  ;
2  1  1  5.74   12.78  11.27  ;
2  1  2  7.65   7.02   11.96  ;
.....
2  1  90 6.58   13.92  9.94   ;
2  2  1  6.01   10.22  9.95   ;
2  2  2  6.22   11.99  7.81   ;
.....
2  2  90 7.98   13.21  12.03  ;
*TraitEnd*
```

The second row includes the indicator strings and the names of the traits. The number of source strings depends on the experimental design. If both environments and replications are taken, a maximum of three strings must be inputted: the first string for environment (Env#), the second string for replication (Rep#) and the last string for genotype (Geno#). You can use whatever strings to express the sources because they are just used to indicate what the numbers are in the columns below them. If the experiment is conducted without environmental factor or replications, the corresponding column must be removed. And also, a semicolon “;” is required at the end of each observation data row.

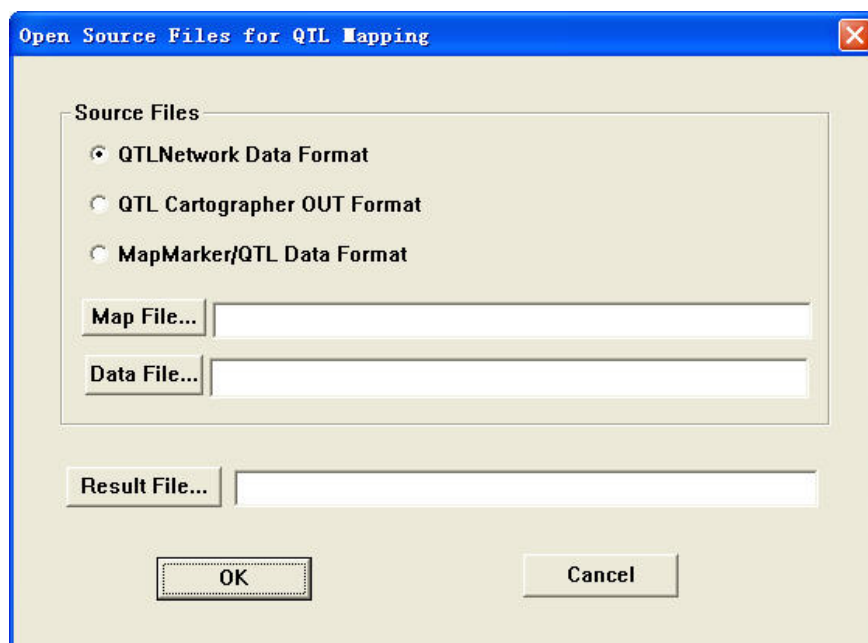
5.1.3 Other acceptable data formats

Our software could also accept the OUT data format of QTL Cartographer (*.map and *.cro) and the data format of MapMaker/QTL (*.map and *.raw). It should be noted that the marker number and order in the RAW file (*.raw) of MapMaker/QTL must be exactly consistent with that in the map file. And the population type after the keyword “TYPE” must have the same specifications as those of QTLNetwork, i.e. “F2” for F2 population, “DH” for DH population, “RI” for Recombination inbred lines, “B1” and “B2” for backcross population to P1 and P2.

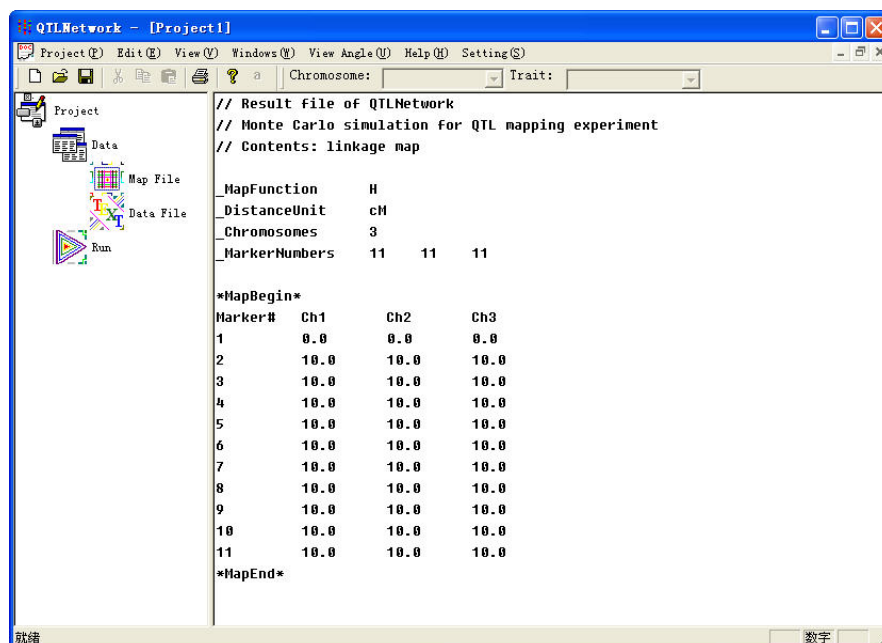
5.2 Create new project with map and data files



5.2.1 Import source data files

The procedure is described below. First click Project -> New to enter the following dialog.



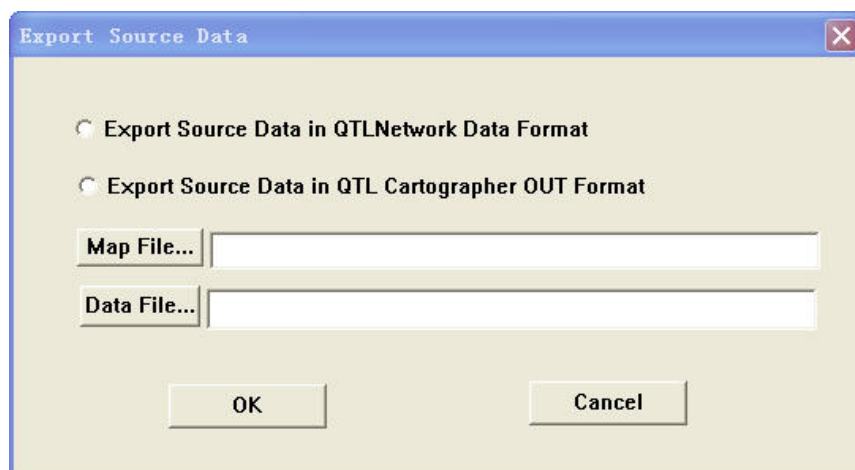
Users can use the Browse... button to load map and data files, and the blank will show the path and filename after the files are chosen. Similarly, users set the result file with the Browse... button (examples of map and data files can be found in the folder “QTLNetwork Installation folder\ SampleData”). Then users press OK.



Users can click the buttons of “Map File”  and “Data File”  in icon tree list in the left to show the text context of files in the view window in the right to check them.

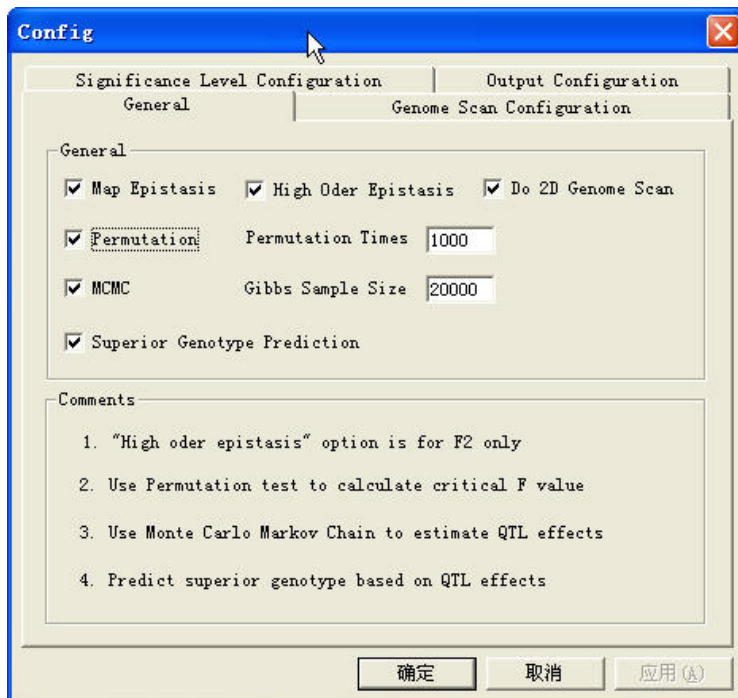
5.2.2 Export source data files

Users could save the source data in other data format by click the Project -> Export Source Data to enter the following dialog. Two types of data formats, QTLNetwork data format and QTL Cartographer OUT data format, are available.

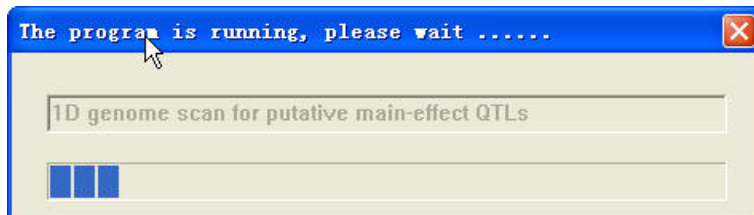


5.2.3 Start computation

After checks, users can click the button of “Run” to start the computation for mapping QTL. And the setting dialog for mapping QTL pops up.

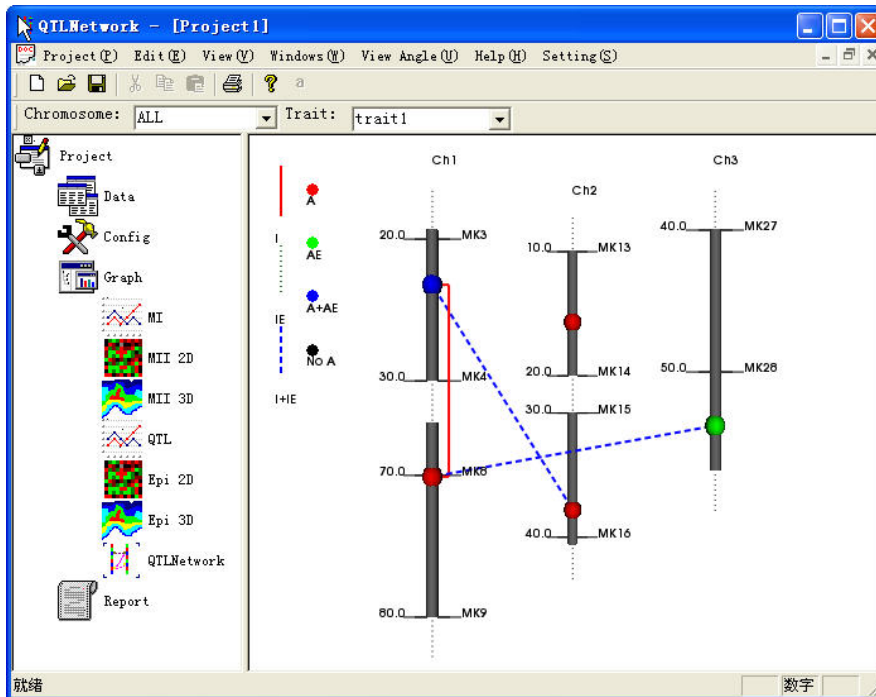



Users can set own parameters for QTL mapping. The detailed information of this Config sheet can be found in Chapter 6. After finishing setting, click the OK button to start the mapping computation.

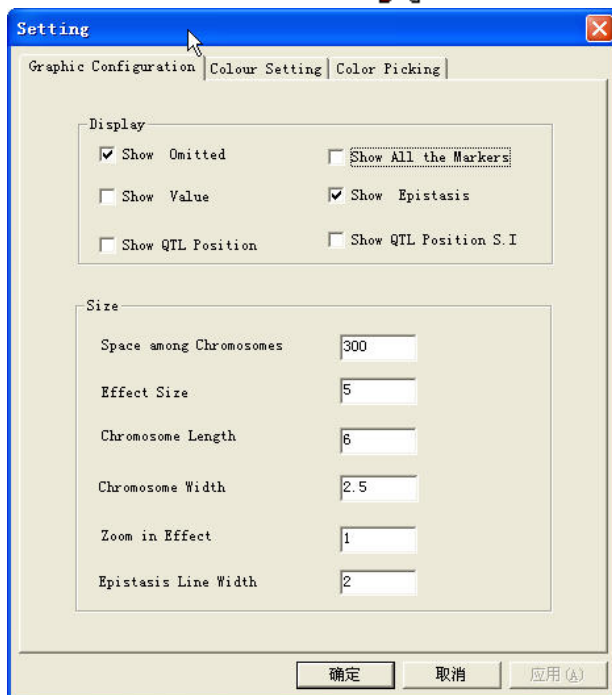


A progress bar pops up to show the users the current process of the computation.


After computation is successfully finished, the software will jump to the picture of QTLNetwork, and all the results will be saved in a file with “qnk” as its extension name. For example, if the project is created by a map file named as “rice.map” and a data files named as “riceph.txt”, the result file will be automatically saved as riceph.qnk. Moreover, users can specify the result file with any other name when you create the project. **Warning! Do not edit the result file (*.qnk), otherwise QTLNetwork may not able to re-open it.**




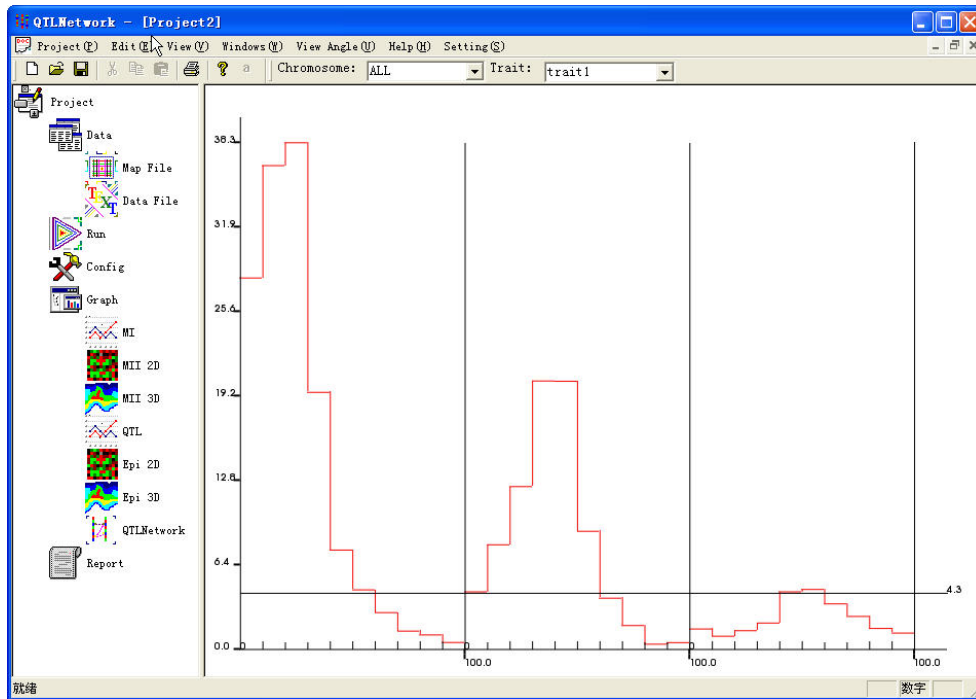
Users can click the Config  Button, and then the Graph Setting dialog pops up,



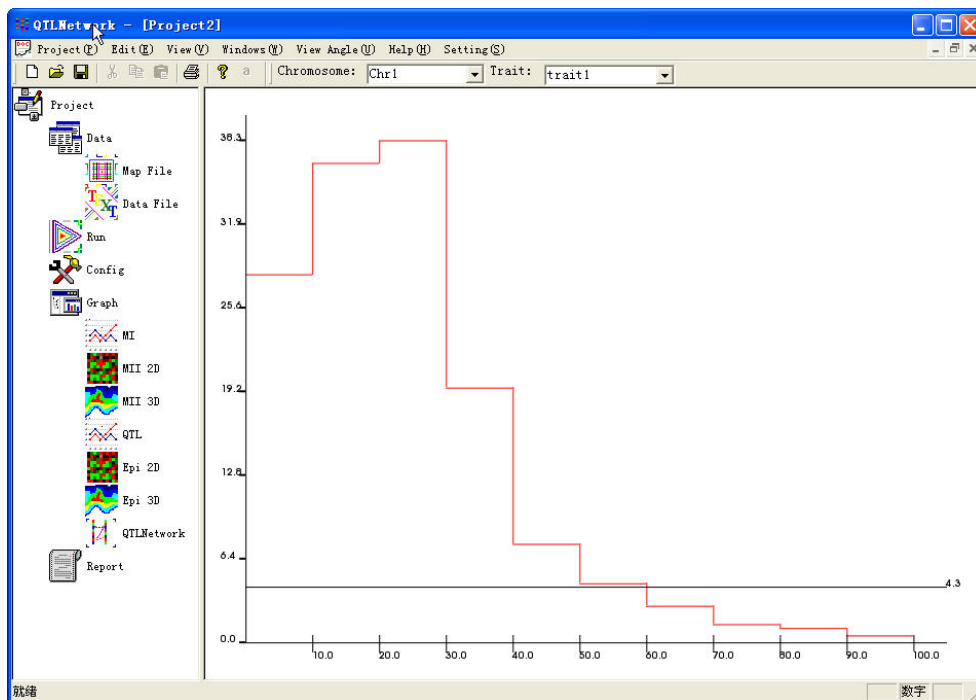
More detailed information about this graph setting dialog will be found in Chapter 7.

Users can click the sub buttons under the Graph button  to reveal different kinds of visualization in the right view windows.

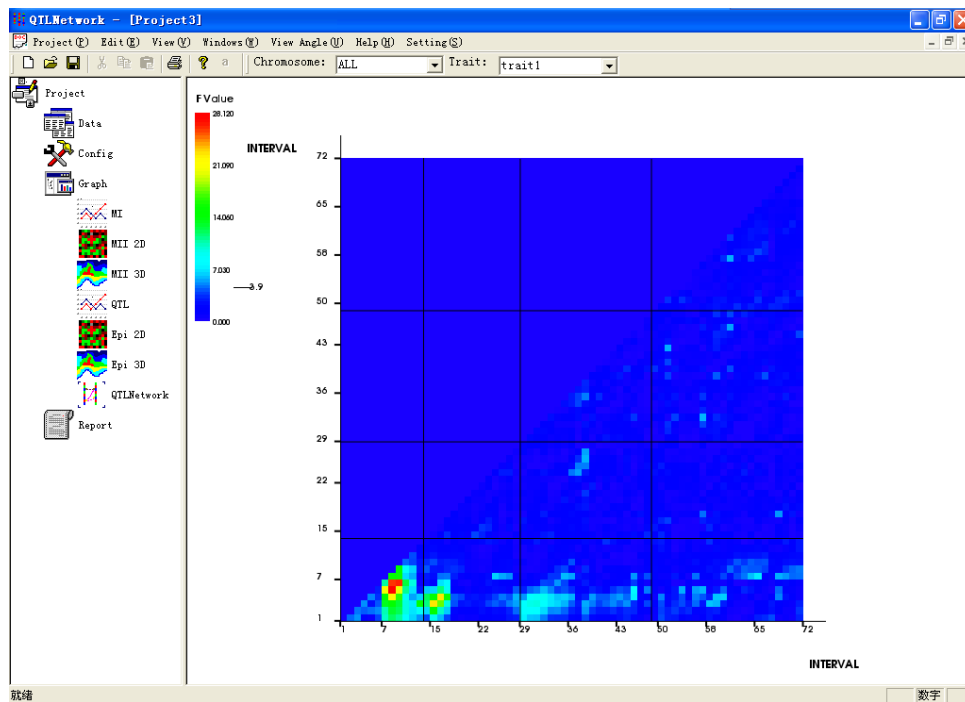
Click button MI  to show the visualization for the 1D test statistics of marker interval analysis.




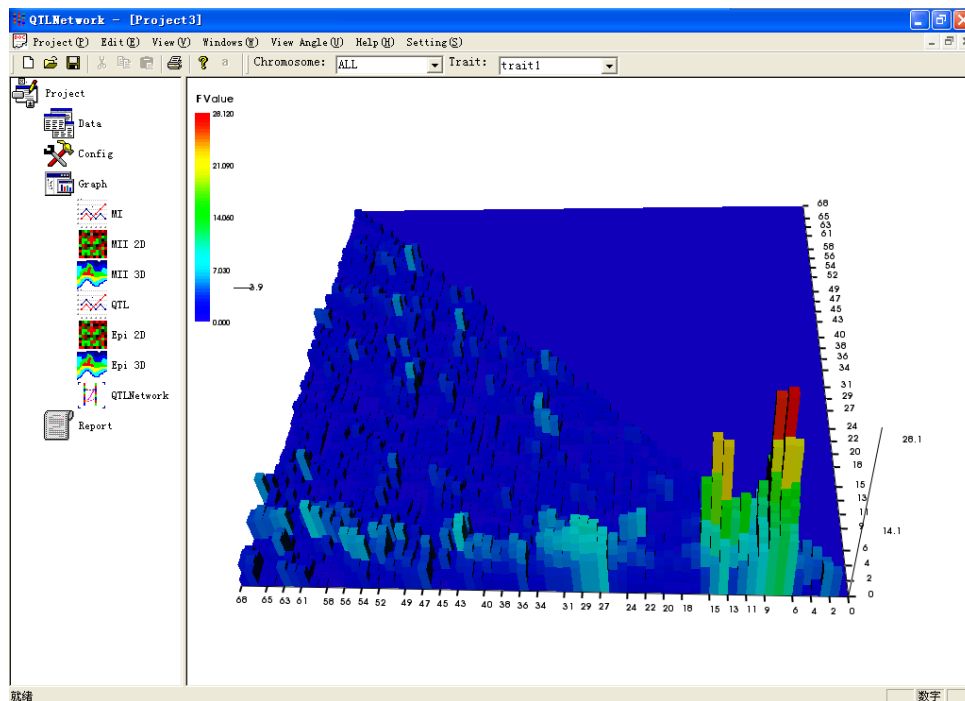
The initial view is about the all chromosomes in triat1. The two list boxes in menu can be used to select the targeted chromosomes and traits.




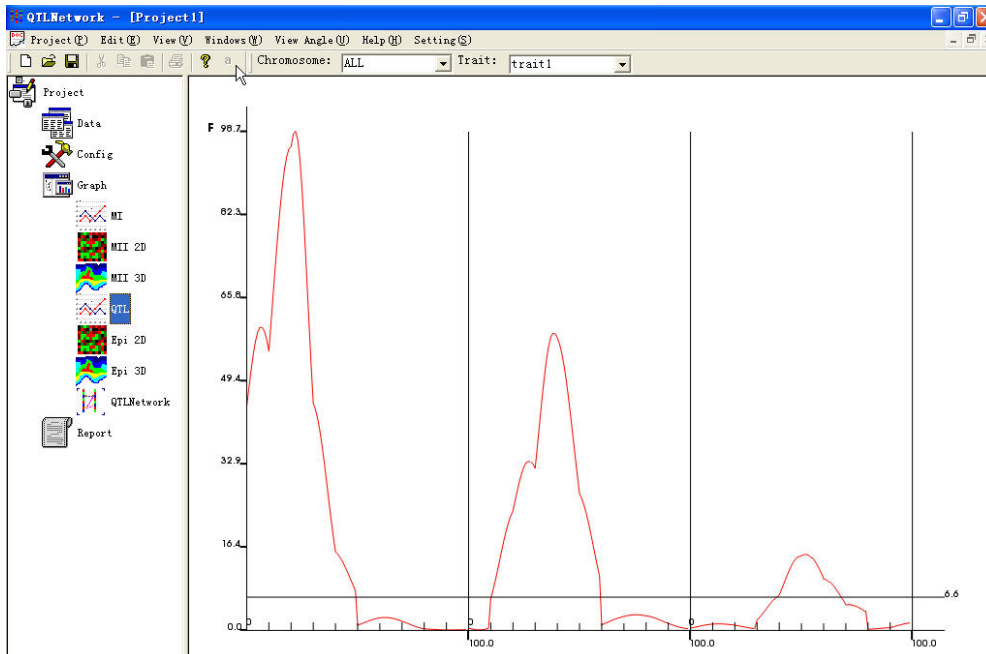
Click the button MII 2D (only available in data with 2D genome scan) to reveal the visualization for 2D statistics of marker interval analysis, where the colorful matrix represents the F value between each pair of intervals, and the color scalar bar provides a continuous mapping from color to F value.




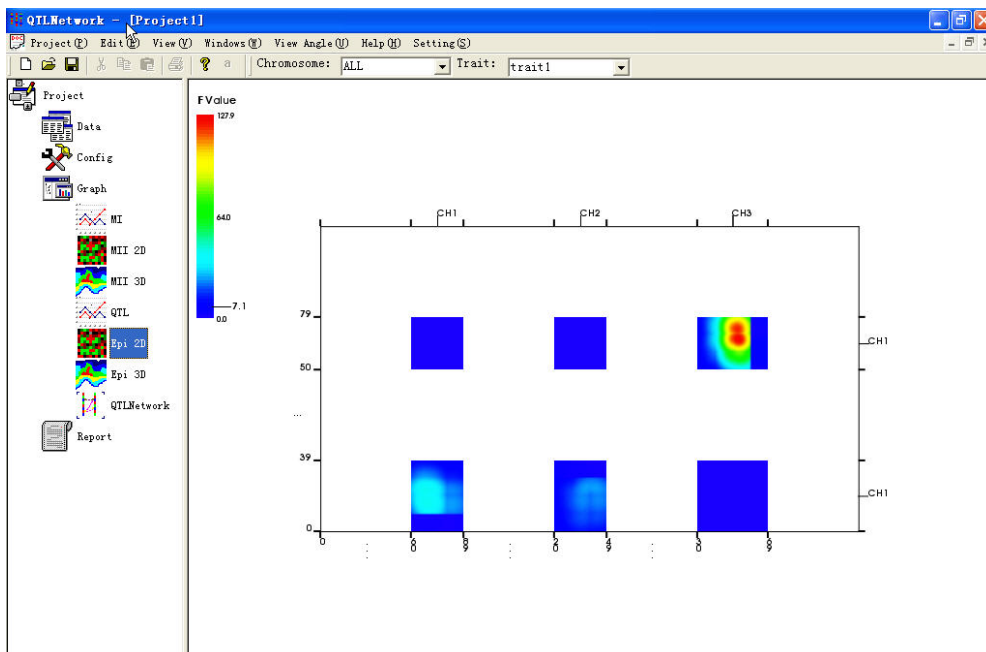
Click MII 3D button  (only available in data with 2D genome scan) to reveal 3D molecular marker interaction plot based on MII 2D visualization, where F value is taken as height.




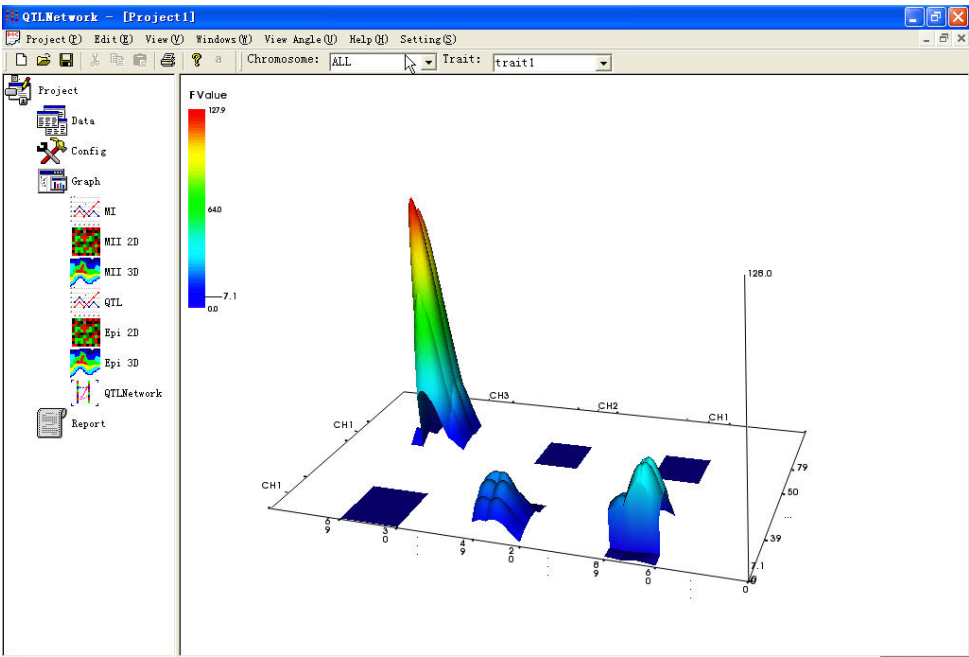
Click the button QTL  to reveal 1D visualization for the test statistics of genome scan for QTL and epistasis. Users can use two list boxes in menu to choose specific chromosomes in targeted traits as MI view.



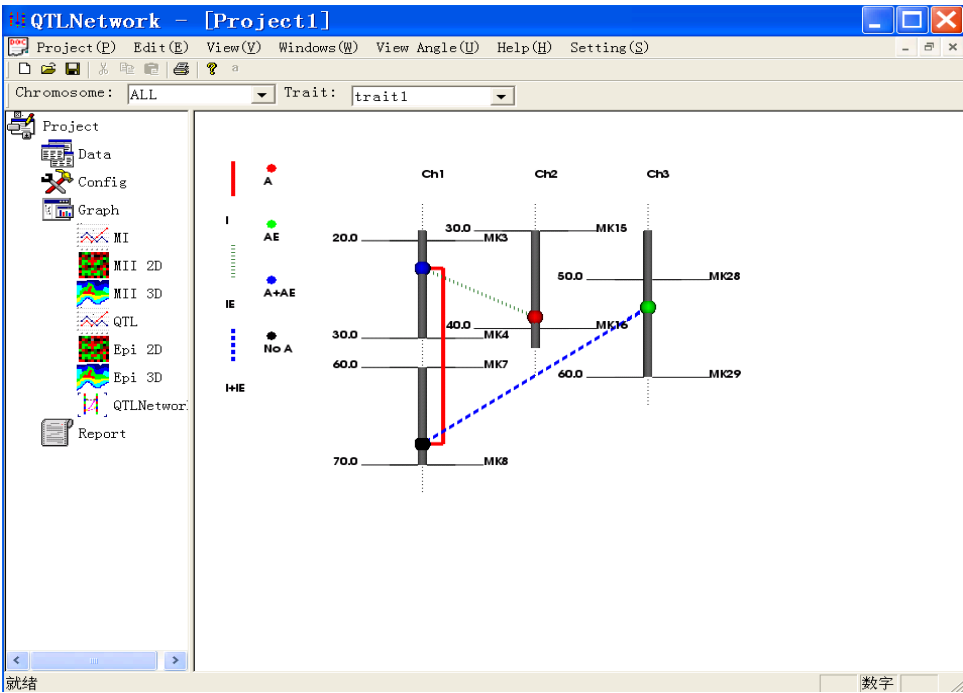
Click the button Epi 2D  (only available in data with 2D genome scan) to reveal 2D visualization for the test statistics of genome scan for QTL and epistasis. The 2D genome scan procedure is only conducted in the chromosome regions nearby the intervals which are involved in interactions with other intervals. Since the testing regions are too trivial in the whole genome, other parts are omitted as small blanks.



Click the button Epi 3D  (only available in data with 2D genome scan) to reveal 3D visualization for the test statistics of genome scan for QTL and epistasis based on Epi 2D visualization, where F value is taken as height.














Click the Button QTLNetwork  to reveal the Graphic presentation of the genetic architecture with QTL and epistasis.




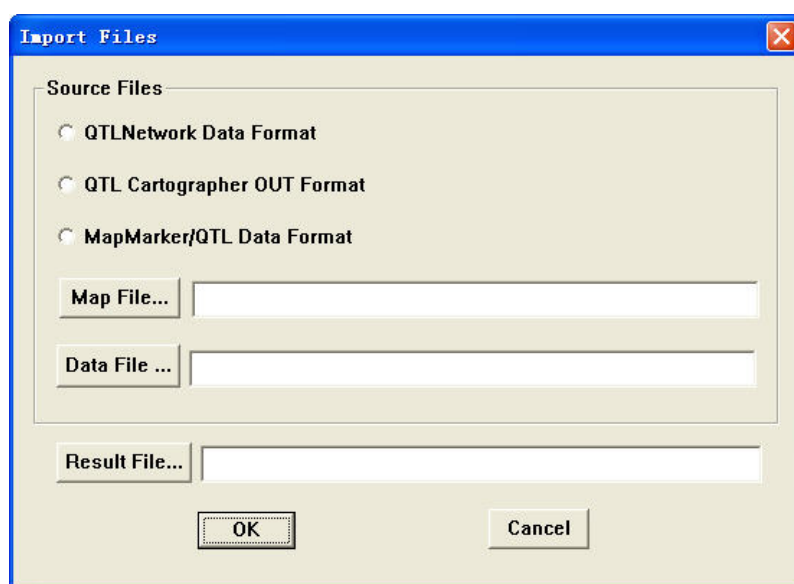
Here is the table for graphic meta system in genetic architecture presentation.

Table: Definition of the graphic meta system for genetic architecture presentation

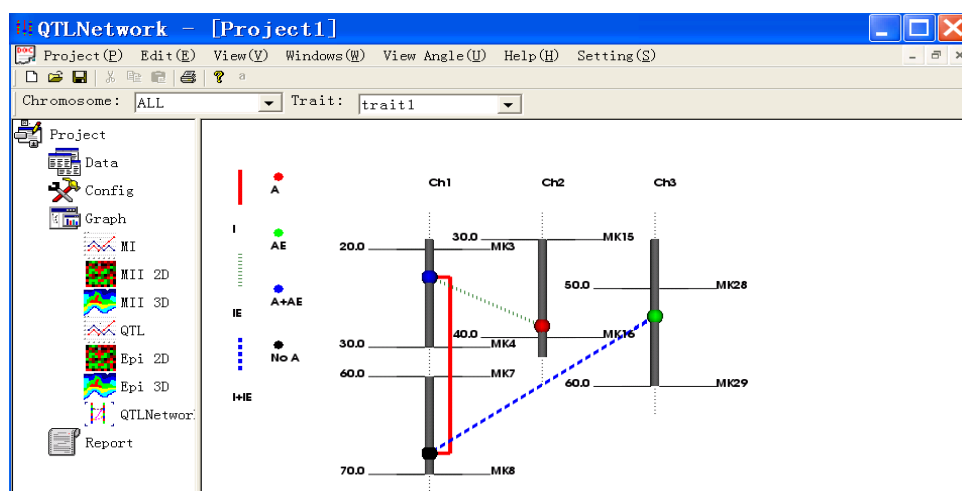
Graphic meta system	Line (Epistasis)	Shape (QTL)	
		Circle	Square
Red	 with only epistatic main effect (I)	 with only additive effect (A)	 with only dominance effect (D)
Green	 with only epistasis \times environment interaction effect (IE)	 with only additive \times environment interaction effect (AE)	 with only dominance \times environment interaction effect (DE)
Blue	 with both I and IE	 with both A and AE	 with both D and DE
Dark	Not available	 with no additive related effect	 with no dominance related effect

5.3 Open the project with map, data and result files

Click Project -> Open, or click the button  in toolbar, and then the open project dialog pops up.



Use the Browse... button to select import files, and all three files including map file, data file and result file should be located correctly. Then press the OK button.



6. Interactive Visualization operations

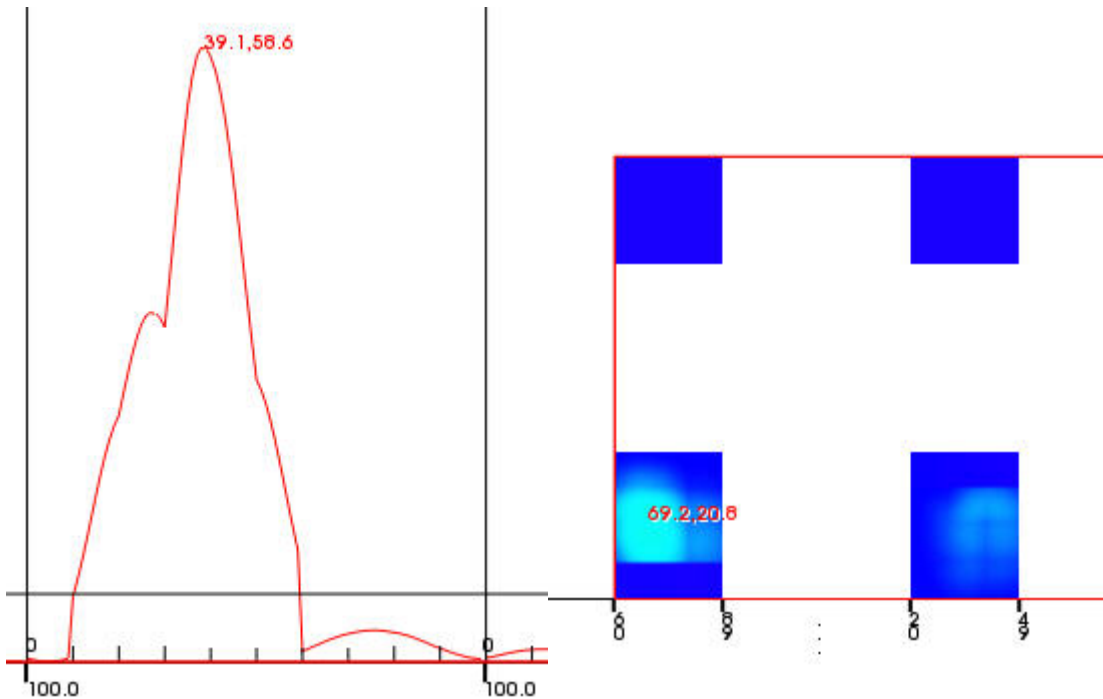
Comfortable and powerful interactive visualization methods with user-friendly interface are provided by QTLNetwork. Most frequently using interactive operations can be easily handled by just only the mouse. Next the details about interactive operations will be described.


Rotation: Press the left key of the mouse and move the mouse arrow.

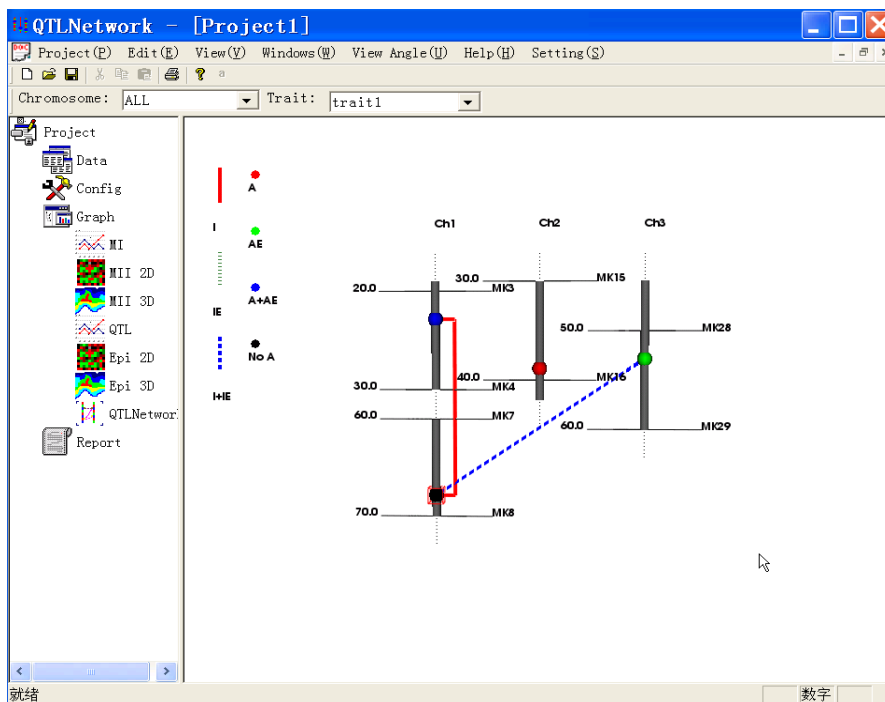
Zoom in and zoom out: Press the right key of the mouse, and move the mouse arrow towards up direction to zoom in and down direction to zoom out.

Translation: Press the middle key of the mouse, and move the mouse arrow to translate the figure.

Pick: Move the mouse arrow to the targeted place, and then press the “P” in keyboard. This operation can be used to show the F value at any genomic position of interest (See the following two examples).

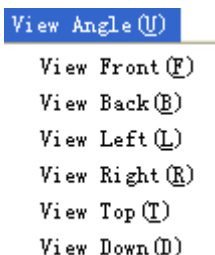


This operation can also be used to show all the epistatic interactions in which one target QTL is involved. This can be accomplished by firstly clicking the config button  to disable the checkbox ☐ Show Epistasis, and then moving the mouse arrow to the target QTL and pressing “P” in the keyboard (See the following example).



View angle

Click “View Angle” in the menu bar. A submenu of view angle will pops up.



View Front – View from the front to the back.

View Back – View from the back to the front.

View Left – View from the left to the right.

View Right – View from the right to the left.

View Top – View from the top to the down.

View Down – View from the down to the top.

7. Config setting in the mapping computation

When new project is loaded, after users click the “Run” button, a config setting sheet will pops up where users can set the parameters for mapping computation. The config setting sheet has four different pages, “General”, “Genome scan configuration”, “Significance level configuration” and “Output configuration”. Let’s start with it one by one.

7.1 General page

Map Epistasis – Choose this option to map both single-locus effect QTL and epistasis, otherwise to map QTL with single-locus effects only.

High Order Epistasis – We define the additive \times dominance (AD), dominance \times additive (DA) and dominance \times dominance (DD) as the high order epistaiss. This option is only available for F2 or IF2 population. Choose this option to map QTL with additive (A) and dominance (D) effects as well as map epistasis with AA, AD, DA and DD effects, otherwise to map epistasis with only AA effect.

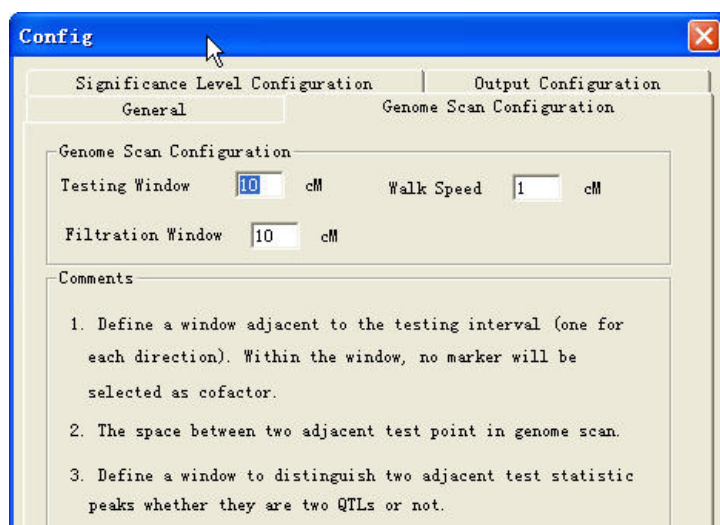
Do 2D Genome Scan – Choose this option to map epistatic QTL with or without single-locus effects. Otherwise the program will only detect the epistatic interaction among QTL with single-locus effect.

Permutation – Choose this option to calculate a critical F value to control the experimental type I error rate by permutation test, otherwise by false discovery rate control.

MCMC – Choose this option to estimate QTL effects by Monte Carlo Markov Chain method, otherwise by mixed linear model approach.

Superior Genotype Prediction – Choose this option to predict superior genotypes based on QTL effects (Ref. Yang J and Zhu J. (2005). Predicting Superior Genotypes in Multiple Environments Based on QTL Effects. Theoretical and Applied Genetics, 110: 1268-1274.)

7.2 Genome scan configuration page

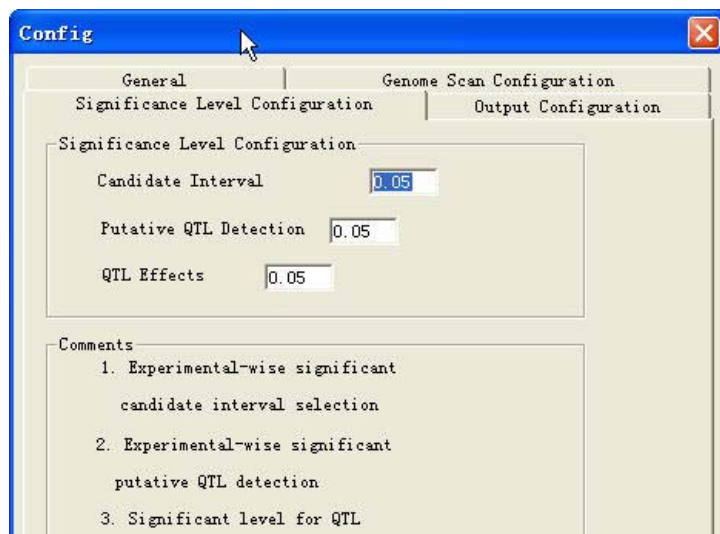


Testing Window – Define a window adjacent to the testing interval (one for each direction). When scan the genome for putative QTL, no marker will be selected as cofactor within the testing window.

Walk Speed – The space between two adjacent test point, when testing for putative QTL along the genome.

Filtration Window – Define a window to distinguish two adjacent test statistic peaks whether they are two QTL or not.

7.3 Significance level configuration page

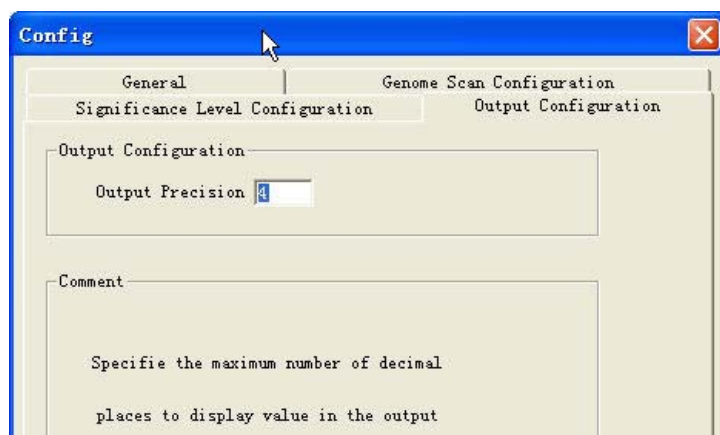


Candidate Interval Selection – The experimental-wise type I error for candidate interval selection.

Putative QTL Detection – The experimental-wise type I error for putative QTL detection.

QTL Effects – The significant level for QTL effects.

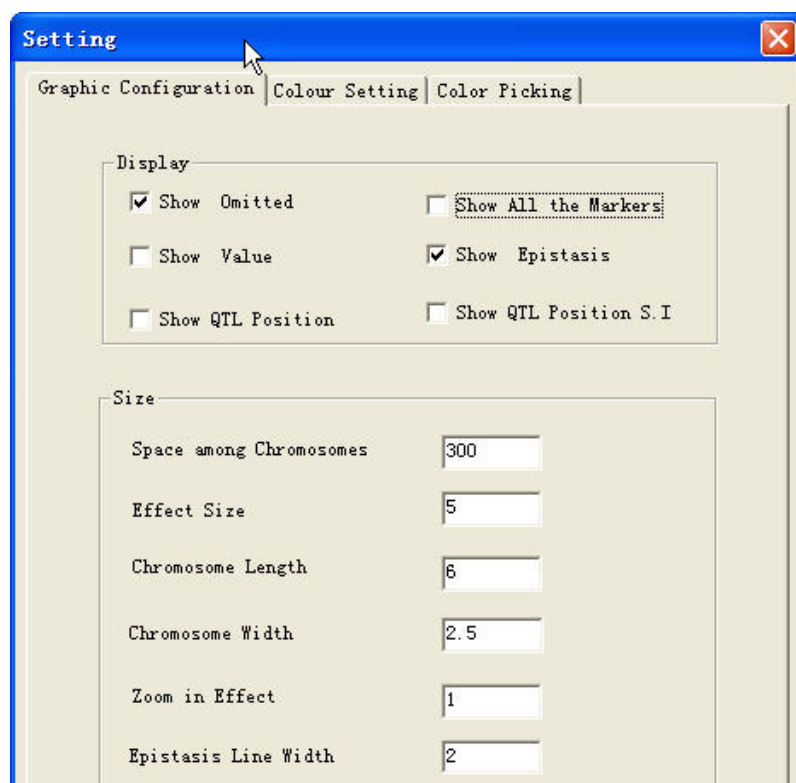
7.4 Output configuration page



Output Precision – The maximum number of decimal places to display value in the output.

8. Graphic configuration

Click the config button  in the left icon list tree. A graphic setting dialog will pop up.



Show Omitted – Choose this option to show the omitted regions of linkage group only for detected QTL, otherwise show the whole linkage group.

Show All the Markers – Choose this option to show all the markers in the linkage group, otherwise only show the markers near QTL.

Show Value – Choose this option to show the effect values of QTL and epistasis.

Show Epistasis – Choose this option to show the epistatic interaction among QTL.

Show QTL Position S.I. – Choose this option to show the support interval of QTL position.

Space among Chromosomes – The distance between two adjacent chromosomes.

Effect Size – The radius of the effect sphere.

Chromosome Length – The length of the chromosome column.

Chromosome Width – The width of the chromosome column.

Zoom in Effect – The scale size of effects in the QTL network graph.

Epistasis Line Width – The width of the epistasis line.

9. Save pictures and reports

9.1 Save pictures

QTLNetwork provides users easy-use interface to save pictures of all the graphs.



There are two different ways of saving pictures, “Save picture” and “Quick SP options”.

Save Picture – Click this item, a normal file dialog appears to help users to save the graph in a targeted directory.

Quick SP – Click this item, the software will automatically save the graph in a picture file, which will be named the data filename plus the serial number. The serial number increases from 1 to N.

9.2 Save and understand the text reports

Click the “Edit” button in the menu bar, and then click “Save Report”. A normal file dialog appears to help users to save the QTL mapping result in text format in a targeted directory.

The report is presented in six parts (if all the options have been chosen in the config dialog before computing) each of which start with a key word.

Part 1 _variance_components (partition the variance components of phenotype variation)

V(G)/V(P)– variance of genetic main effects divided by phenotypic variance;

V(E)/V(P)– variance of environmental effects divided by phenotypic variance;

V(GE)/V(P)– variance of genotype-by-environment interaction effects divided by phenotypic variance;

V(e) /V(P)– variance of residual effects divided by phenotypic variance;

V(p)– phenotypic variance;

V(A)/V(P)– variance of additive effects divided by phenotypic variance;

V(AE)/V(P)– variance of additive-by-environment interaction effects divided by phenotypic variance;

V(D)/V(P)– variance of dominance effects divided by phenotypic variance;

V(DE)/V(P)– variance of dominance-by-environment effects divided by phenotypic variance;

V(I)/V(P)– variance of epistatic effects divided by phenotypic variance;

V(IE)/V(P)– variance of epistasis-by-environment effects divided by phenotypic variance;

V(A+AA)/V(P)–variance of additive and additive by additive effects divided by phenotypic variance;

V(AE+AAE)/V(P)–variance of additive-by-environment and aa by environment interaction effects divided by phenotypic variance.

Part 2 _heterosis (mid-parent and better parent heterosis in each environment)

Hr– two kinds of heterosis, mid-parent heterosis and better parent heterosis;

e_x– heterosis in x-th environment (only available in data with multiple environments);

general– the general heterosis (only available in data without environments);

mid-parent– mid-parent heterosis;

better-parent– better-parent heterosis.

Part 3 _QTL (mapping results of single-locus effect QTL)

QTL– QTL is named with the relevant chromosome and the marker intervals. For example, if a QTL is named as 3-6, it means that this QTL locates at the 6th marker interval of the 3th chromosome.

Interval – The flanking markers of QTL.

Position – The distance between QTL and the first marker of the relevant chromosome.

Range – The support interval of QTL position.

A – The estimated additive effect.

D –The estimated dominance effect.

AE – The predicted additive by environment interaction effect.

DE –The predicted dominance by environment interaction effect.

SE and P-Value –The standard error of estimated or predicted QTL effect and P-value.

Part 4 _QTL_heritability (heritabilities of QTL effects)

h²(a)– The heritability of additive effect.

h²(d)– The heritability of dominance effect.

h²(ae)– The heritability of additive by environment interaction effects.

h²(de)– The heritability of dominance by environment interaction effects.

Part 5 _QTL_heterosis (heterosis of QTL effects)

HMP(q)– The general mid-parent heterosis due to dominance effects.

HMP(qex)– The mid-parent heterosis due to dominance by environment effects in the x-th environment.

HBP(q)–The general better parent heterosis due to dominance effects.

HBP(qex)–The better parent heterosis due to dominance by environment effects in the x-th environment.

Part 6 _epistasis (mapping results of epistasis)

QTL_i and QTL_j– The two QTL involved in epistatic interaction.

interval_i– The flanking markers of QTL_i.

position_i– The distance between QTL_i and the first marker of the relevant chromosome

range_i– The position support interval of QTL_i.

interval_j– The flanking markers of QTL_j.

position_j– The distance between QTL_j and the first marker of the relevant chromosome

range_j– The position support interval of QTL_j.

AA – The estimated additive by additive effect.

AD – The estimated additive by dominance effect.

DA –The estimated dominance by additive effect.

DD – The estimated dominance by dominance effect.

AAE – The predicted aa by environment interaction effect.

ADE – The predicted ad by environment interaction effect.

DAE – The predicted da by environment interaction effect.

DDE –The predicted dd by environment interaction effect.

SE and P-Value –The standard error of estimated or predicted QTL effect and P-value.

Part 7 _epistasis_heritability (heritabilities of epistatic effects)

$h^2(aa)$ – The heritability of additive by additive effect.

$h^2(ad)$ – The heritability of additive by dominance effect.

$h^2(da)$ – The heritability of dominance by additive effect.

$h^2(dd)$ – The heritability of dominance by dominance effect.

$h^2(aae)$ – The heritability of aa by environment interaction effects.

$h^2(ade)$ – The heritability of ad by environment interaction effects.

$h^2(dae)$ – The heritability of da by environment interaction effects.

$h^2(ade)$ – The heritability of dd by environment interaction effects.

Part 8 _epistasis_heterosis (heterosis of epistatic effects)

Hr– Heterosis due to epistatic effects. As mid-parent heterosis and better parent heterosis due to epistatic effects are the same, Hr is short for mid-heterosis or better parent heterosis.

Hr(qq)– The general heterosis due to epistatic effects.

Hr(qqex)–The heterosis due to epistasis by environment effects in the x-th environment.

Part 9 _genotype_value (genetic effects of the two parents, the F1 hybrid and the predicted superior genotypes)

G– general genetic effects of P1, P2 and F1 hybrid as well as the predicted general superior lines and general superior hybrids.

G+GE– total genetic effects of P1, P2 and F1 hybrid in a specific environment as well as the predicted superior lines and superior hybrids in that environment.

The notation (+) and (–) denote the superior genotypes are predicted to get the maximum and minimum genetic effects, respectively.

Part 10 _superior_genotype (QTL genotypes of the predicted superior genotypes)

G– specifies the general genetic effects of P1, P2 and F1 hybrid as well as the predicted general superior genotypes.

G+GE– specifies the total genetic effects of P1, P2 and F1 hybrid in a specific environment as well as the predicted superior genotype in that environment.

Part 11 _individual_genotype_value (genetic effects of different genotypes)

In this part, program presents sorted individual genetic effects with maximum and minimum estimation, of which scale is determined by the decimal fraction after keyword “_Genotypes”. The estimates are divided into several parts, including general individual genetic effects estimation and individual genetic effects prediction in each specific environment. In each part, the results include individual genetic effects estimation (G) or prediction in each specific environment (G+GE), and genetic effects estimates of individual QTLs and epistatic QTLs where two QTLs are connected with “^”.

```

_Population      DH
_Genotypes       99 0.1
_Observations    198
_Environments     yes
_Replications     yes
_TraitNumber      4
_TotalMarker      54
_MarkerCode      P1=1    P2=2    F1=3    F1P1=4  F1P2=5

```

For instance, 0.1 is typed after the keyword “Genotypes”, as a result, 10% largest individual genetic effects and 10% smallest individual genetic effects will be presented in the result file.

```

_general
_for_10%_highest_value
Entry      G          1-15          2-12          3-20          2-12^3-20      2-9^3-7
64          29.2141      10.2334      4.6603      7.7713      2.5815      3.9676
97          26.4951      10.2334      2.7094      8.0333      1.5514      3.9676
48          25.1692      10.2334      4.6603      8.0333      2.6686      -0.4263
42          24.4960      5.1661      4.6603      8.0333      2.6686      3.9676
73          24.1469      5.1661      4.6603      7.7713      2.5815      3.9676
32          21.8212      10.2334      4.6603      7.7713      2.5815      -3.4252
39          21.2788      10.2334      4.6603      7.7713      2.5815      -3.9676
40          21.2788      10.2334      4.6603      7.7713      2.5815      -3.9676
28          18.3514      10.0249      2.7094      8.0333      1.5514      -3.9676

_for_10%_lowest_value
Entry      G          1-15          2-12          3-20          2-12^3-20      2-9^3-7
45          -24.2260      -10.2334      -4.6603      -8.0333      2.6686      -3.9676
65          -24.0511      -10.2334      -4.6603      -7.7713      2.5815      -3.9676
13          -21.0764      -10.2334      2.7094      -8.0333      -1.5514      -3.9676
19          -19.8936      -10.2334      4.6603      -7.7713      -2.5815      -3.9676
6          -19.6571      -10.2334      -4.6603      -7.7713      2.5815      0.4263
74          -19.6571      -10.2334      -4.6603      -7.7713      2.5815      0.4263
93          -18.9838      -5.1661      -4.6603      -7.7713      2.5815      -3.9676
43          -16.7013      -10.2334      4.6603      -8.0333      -2.6686      -0.4263
90          -16.7013      -10.2334      4.6603      -8.0333      -2.6686      -0.4263

```

As shown in the figure above, 9 largest individual genetic effects and 9 smallest individual

genetic effects are listed, and the first part is the general individual genetic effects (G) followed with genetic effects estimates of three individual QTLs (1-15, 2-12, and 3-20) and two epistatic QTLs ($2-12^3-20$ and $2-9^3-7$)

The second part is the individual genetic effects in the first environment (G+GE1) followed with genetic effects estimates of three individual QTLs and two epistatic QTLs in the first environment.

<u>_environment_1</u>						
<u>_for_10%_highest_value</u>						
Entry	G+GE1	1-15	2-12	3-20	$2-12^3-20$	$2-9^3-7$
64	29.2141	10.2334	4.6603	7.7713	2.5815	3.9676
97	26.4951	10.2334	2.7094	8.0333	1.5514	3.9676
48	25.1692	10.2334	4.6603	8.0333	2.6686	-0.4263
42	24.4960	5.1661	4.6603	8.0333	2.6686	3.9676
73	24.1469	5.1661	4.6603	7.7713	2.5815	3.9676
32	21.8212	10.2334	4.6603	7.7713	2.5815	-3.4252
39	21.2788	10.2334	4.6603	7.7713	2.5815	-3.9676
40	21.2788	10.2334	4.6603	7.7713	2.5815	-3.9676
28	18.3514	10.0249	2.7094	8.0333	1.5514	-3.9676
<u>_for_10%_lowest_value</u>						
Entry	G+GE1	1-15	2-12	3-20	$2-12^3-20$	$2-9^3-7$
45	-24.2260	-10.2334	-4.6603	-8.0333	2.6686	-3.9676
65	-24.0511	-10.2334	-4.6603	-7.7713	2.5815	-3.9676
13	-21.0764	-10.2334	2.7094	-8.0333	-1.5514	-3.9676
19	-19.8936	-10.2334	4.6603	-7.7713	-2.5815	-3.9676
6	-19.6571	-10.2334	-4.6603	-7.7713	2.5815	0.4263
74	-19.6571	-10.2334	-4.6603	-7.7713	2.5815	0.4263
93	-18.9838	-5.1661	-4.6603	-7.7713	2.5815	-3.9676
43	-16.7013	-10.2334	4.6603	-8.0333	-2.6686	-0.4263
90	-16.7013	-10.2334	4.6603	-8.0333	-2.6686	-0.4263

The third part is the individual genetic effects in the second environment (G+GE2) followed with genetic effects estimates of three individual QTLs and two epistatic QTLs in the first environment.

<u>_environment_2</u>						
<u>_for_10%_highest_value</u>						
Entry	G+GE2	1-15	2-12	3-20	$2-12^3-20$	$2-9^3-7$
64	29.2141	10.2334	4.6603	7.7713	2.5815	3.9676
97	26.4951	10.2334	2.7094	8.0333	1.5514	3.9676
48	25.1692	10.2334	4.6603	8.0333	2.6686	-0.4263
42	24.4960	5.1661	4.6603	8.0333	2.6686	3.9676
73	24.1469	5.1661	4.6603	7.7713	2.5815	3.9676
32	21.8212	10.2334	4.6603	7.7713	2.5815	-3.4252
39	21.2788	10.2334	4.6603	7.7713	2.5815	-3.9676
40	21.2788	10.2334	4.6603	7.7713	2.5815	-3.9676
28	18.3514	10.0249	2.7094	8.0333	1.5514	-3.9676
<u>_for_10%_lowest_value</u>						
Entry	G+GE2	1-15	2-12	3-20	$2-12^3-20$	$2-9^3-7$
45	-24.2260	-10.2334	-4.6603	-8.0333	2.6686	-3.9676
65	-24.0511	-10.2334	-4.6603	-7.7713	2.5815	-3.9676
13	-21.0764	-10.2334	2.7094	-8.0333	-1.5514	-3.9676
19	-19.8936	-10.2334	4.6603	-7.7713	-2.5815	-3.9676
6	-19.6571	-10.2334	-4.6603	-7.7713	2.5815	0.4263
74	-19.6571	-10.2334	-4.6603	-7.7713	2.5815	0.4263
93	-18.9838	-5.1661	-4.6603	-7.7713	2.5815	-3.9676
43	-16.7013	-10.2334	4.6603	-8.0333	-2.6686	-0.4263
90	-16.7013	-10.2334	4.6603	-8.0333	-2.6686	-0.4263

Part 12 _configuration_setting_in_computation

This section presents the configuration setting in the computation, including the general setting (epistasis analysis, permutation times, Gibbs sampling size, and superior genotype prediction), significance level configuration (significance level of candidate interval and interval pairs, putative QTL and epistatic QTLs detection, and QTL effects), and genome scan configuration (size of testing window and filtration window, length of walk speed).