# **QTLNetwork-2.0 User Manual**

Software for Mapping QTL with Epistatic and QE Interaction Effects



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### 1. 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 Tookit). 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.

### 2 Installing QTLNetwork

The software is freely available from the URL <u>http://ibi.zju.edu.cn/software/qtlnetwork/</u>. 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.

ser Informa	ition			17 mil
Enter you	r user informat	ion and click No	ext to continue.	
Name:				
Your Name	í			
Company:				
Your Comp	any			

Please read the information below and clic continue.	c Next to
QTLNetwork-2.0	~
QTLNetwork-2.0 is user-friendly computer mapping quantitative trait loci (QTL) with and QE interaction effects in DH, RI, BC1, BCxFy populations, and for graphical press mapping results. The software is programmed language under Microsoft Visual C++ 6.0 er with Microsoft Windows operating systems, 95/96, NT, 2000, XP, 2003server. A new ver is under developing, and its functions will include linkage group construction and mar breeding. This software can be freely redistribu purpose and under the condition of no char	software for epistatic effects BC2, F2, IF2 and ntation of QTL d by C++ programming vironment. It works including Windows sion of QTLNetwork l be extended to ker-assisted virtual ted with non-commercial ges to the software and

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

\_4\_

stallation Folder	11 Fatters
Select an installation folder and o continue.	:lick Next to
The software will be installed in t to a different folder, either type browse for an existing folder.	the folder listed below. To install in a new path, or click Change to
Install QTLNetwork to:	
C:\Program Files\QTLNetwork	Change
Space required on drive:	24.5 MB
	21085 MB
Space available on selected	
Space available on selected	

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.

QTLNetwork Setup	
<b>Ready to Install</b> You are now ready to install QTLNetwork 2.0.	
The installer now has enough information to install QTLNe your computer.	etwork on
WThe following settings will be used:	
Install folder: C:\Program Files\QTLNetwork	
Shortcut folder: QTLNetwork	
Please click Next to proceed with the installation.	
< Back Next >	Cancel
🤇 Back Next >	Cancel

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

🙀 Installing Files	X
Installing Fares The program files are being installed.	
Flease wait while the necessary files are installed.	
Installing	
C:\Program Files\QTLNetwork\vtkIO.dll	
(****************************	
	Cancel

Wait till the installation procedure finish.

🛱 QTLNetwork Setup	
	QTLNetwork Installed Successfully Setup is complete and your software is now installed. Thank you for choosing QTLNetwork 2.0. Please click Finish to exit this installer.
	Finish

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

## **3 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.

### 4 Starting with QTLNetwork

#### 4.1 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.

#### 4.1.1 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 table) 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\*

Mai	ker# Ch	1 Cł	n2	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 third 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.

#### 4.1.2 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	$P1 \times B1B1$	<i>B</i> 2 <i>B</i> 1	<i>B2B</i> 1
$ \otimes$		$ \otimes$	double-haploid
F4	<i>B</i> 1 <i>B</i> 1 <i>B</i> 1	B2B1F	<i>B2B1D</i>

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\*

1	Mk	1	Mk	2	Mk	3	Mk	4	Mk5	Mk6	Mk7	Mk8	Mk9;
1	1	1	2	2	2	2	1	1	;				
1	1		1	1	2	2	2	2	;				
2		2	1	1	1	1	2	2	?				
2	2	2	2		1	1		1	;				
1	1	2	2	2	2	2	1	1	;				
arke	rEnd	*											
	1 1 2 2 1 arket	<ul> <li>Mk</li> <li>1</li> <li>1</li> <li>1</li> <li>2</li> <li>.</li> <li>.</li> <li>2</li> <li>2</li> <li>2</li> <li>1</li> <li>1</li> </ul>	Mk1       1     1       1     1       1     1       2     .       2     .       2     2       .       2     2       1     1       2     2       2     2       1     1       2     2       2     1       1     2	i     Mk1     Mk       1     1     1     2       1     1     .     1       2     .     2     1       .     .     .     .       2     2     2     2       1     1     2     2       .     .     .     .       .     .	Mk1     Mk2       1     1     1     2     2       1     1     .     1     1       2     .     2     1     1       .     .     .     1     1       .     .     .     .     .       1     1     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .       .     .     .     .     .	i     Mk1     Mk2     Mk       1     1     1     2     2     2       1     1     .     1     1     2       2     .     2     1     1     1       .     .     .     .     1     1       .     .     .     .     1     1       1     1     2     2     .     1       1     1     2     2     .     1       1     1     2     2     2     2	Mk1     Mk2     Mk3       1     1     1     2     2     2       1     1     1     2     2     2       1     1     .     1     1     2     2       2     .     2     1     1     1     1       .     .     .     .     1     1     1       .     .     .     .     .     1     1       1     1     2     2     .     1     1       1     1     2     2     2     2     2	i       Mk1       Mk2       Mk3       Mk         1       1       1       2       2       2       1         1       1       1       2       2       2       1         1       1       .       1       1       2       2       2         2       .       2       1       1       1       1       2         .       .       .       1       1       1       2       2         .       .       .       .       .       .       .       .         1       1       2       2       .       .       1       1       .         1       1       2       2       2       .       1       1       .         .       .       .       .       .       .       .       .       .         .       .       .       .       .       .       .       .       .         .       .       .       .       .       .       .       .       .       .         .       .       .       .       .       .       .       .	i       Mk1       Mk2       Mk3       Mk4         1       1       1       2       2       2       1       1         1       1       1       2       2       2       1       1         1       1       .       1       1       2       2       2       2         2       .       2       1       1       1       2       2       2         .       .       .       1       1       1       2       2       2         .       .       .       1       1       1       1       2       2         .       .       .       .       .       1       1       .       1         1       1       2       2       .       .       1       1       .       1         1       1       2       2       .       .       1       1       .       1         arkerEnd*       .       .       .       .       .       .       .       .	I       Mk1       Mk2       Mk3       Mk4       Mk5         1       1       1       2       2       2       1       1       ;         1       1       1       1       2       2       2       1       1       ;         1       1       .       1       1       2       2       2       ;         2       .       2       1       1       1       2       2       ;         2       2       2       2       .       1       1       2       2       ;         2       2       2       2       .       1       1       .       1       ;         1       1       2       2       2       .       1       1       .       .       .         1       1       2       2       2       .       1       1       .       .       .       .         arkerEnd*       .       .       .       .       .       .       .       .	I       Mk1       Mk2       Mk3       Mk4       Mk5       Mk6         1       1       1       2       2       2       1       1       ;         1       1       1       1       2       2       2       1       1       ;         1       1       .       1       1       2       2       2       ;         2       .       2       1       1       1       2       2       ;         .       .       .       1       1       1       .       1       ;         .       .       .       .       1       1       .       .       1       ;         .       .       .       .       .       .       .       .       .       .         .       .       .       .       .       .       .       .       .       .       .       .         .	i       Mk1       Mk2       Mk3       Mk4       Mk5       Mk6       Mk7         1       1       1       2       2       2       1       1       ;         1       1       1       1       2       2       2       1       1       ;         1       1       1       1       2       2       2       ;       ;         2       .       2       1       1       1       2       2       ;       ;         2       2       2       2       .       1       1       .       1       ;         2       2       2       2       .       1       1       .       1       ;         1       1       2       2       2       1       1       ;       .         1       1       2       2       2       1       1       ;       .         arkerEnd*       .       .       .       .       .       .       .       .	i       Mk1       Mk2       Mk3       Mk4       Mk5       Mk6       Mk7       Mk8         1       1       1       2       2       2       1       1       ;         1       1       1       1       2       2       2       2       ;         1       1       1       1       2       2       2       ;       ;         2       .       2       1       1       1       2       2       ;         .       .       .       1       1       1       2       2       ;         .       .       .       1       1       .       1       ;       .         .       .       .       .       .       .       .       .       .         .       .       .       .       .       .       .       .       .         .       .       .       .       .       .       .       .       .         .       .       .       .       .       .       .       .       .         .       .       .       .       .       .       .

#### 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	;

-11-

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	<b>v</b> #	Rep	o# Ger	no# Tra	it_1 Tra	it_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.

#### 4.1.3 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 MapMarker/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.

### 4.2 Create new project with map and data files

#### 4.2.1 Import source data files

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

Source Files	
• QTLNetwork Data Format	
© QTL Cartographer OUT Format	
C MapMarker/QTL Data Format	
Map File	
Data File	
Result File	
,	
· · · · · · · · · · · · · · · · · · ·	

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.

QTLNetwork - [Project	:1]				
🕎 Project(P) Edit(E) View()	() Windows(W)	View Ang	le(U) Help(H)	Setting(S)	_ @ ×
🗅 🗀 🖬   X 🖻 💼 🎒	🤋 a    C	Chromosome	*	- Trait:	
Project Project Data Map File Data File Run	// Result // Monte // // Content MapFunct: Distance Distance Chromosou MarkerNum *MapBegin Marker# 1	<pre>hromosome file of Carlo si ts: link unit mes mbers * Ch1 8.8</pre>	CTLNetwork mulation for age map H CM 3 11 11 Ch2 0.8	• QTL mapping experiment 11 Ch3 0.0	
	2 3 4 5 6 7 8 9 10 11 *MapEnd*	10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0	10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0	10.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0	
就绪					数字

Users can click the buttons of "Map File" in 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.

### 4.2.2 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.

: Data Format	
rapher OUT Format	
	_
	-
Cancel	
	c Data Format rapher OUT Format Cancel

### 4.2.3 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.

pienticance rev	el Configuration	Output Configuration
General	Genom	ne Scan Configuration
meral		
7 Map Epistasis	🔽 High Oder Epistas	is 🔽 Do 2D Genome Scan
7 Permutation	Permutation Times 🏼	000
Z MCMC	Gibbs Sample Size 💈	20000
omments		
1. "High oder e	pistasis" option is fo	r F2 only
2. Use Permutat:	ion test to calculate	critical F value
3. Use Monte Car	rlo Markov Chain to es	timate QTL effects
4. Predict super	rior genotype based on	QTL effects

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.

The program is running, please wait	
12	
1D genome scan for putative main-effect QTLs	

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.



the click Users can

Config 📌 Button, and then the Graph Setting dialog pops up,

Setting	
Graphic Configuration Colour Sett	ing   Color Picking
Display	
🔽 Show Omitted	Show All the Markers
☐ Show Value	🔽 Show Epistasis
🕅 Show QTL Position	TShow QTL Position S.I
Size	
Space among Chromosomes	300
Effect Size	5
Chromosome Length	6
Chromosome Width	2.5
Zoom in Effect	1
Epistasis Line Width	2
[	<b>确定 取消</b> 应用 ( <u>A</u> )

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 is 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 5 (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 is 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 **EFF** (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 [12] 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	Lina (Enistasis)	Shape (QTL)		
meta system	Line (Epistasis)	Circle	Square	
Dod	— with only epistatic	• with only additive	with only dominance	
Keu	main effect (I)	effect (A)	effct (D)	
	with only epistasis $\times$	🔶 with only additive ×	with only dominance ×	
Green	environment interaction effect	environment interaction	environment interaction	
	(IE)	effect (AE)	effect (DE)	
Blue	with both I and IE	with both A and AE	with both D and DE	
Dark	Not available	• with no additive	■ with no dominance	
	not available	related effect	related effect	

### 4.3 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.

port Files		
Source Files		
C QTLNetwork Data Format		
© QTL Cartographer OUT Format		
C MapMarker/QTL Data Format		
Map File		
Data File		1
Result File		_
,		
OK	Cancel	

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.



### **5** 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 epitatic interactions in which one target QTL is involved. This can be accomplished by firstly clicking the config button  $\frac{1}{2}$  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 Angle (U) View Front (F) View Back (B) View Left (L) View Right (R) View Top (T) View Down (D)

**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.

### 6 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.

## 6.1 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. <u>Yang J and Zhu J. (2005)</u>. <u>Predicting Superior Genotypes in Multiple</u> Environments Based on QTL Effects. Theoretical and Applied Genetics, 110: 1268-1274.)

## 6.2 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.

## **6.3 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.

### 6.4 Output configuration page

onfig	k		
Gene	ral	Geno	me Scan Configuration
Significa	nce Level Configu	ration	Output Configuration
-Output Conf Output	iguration Precision 🚺		
Comment			
Specifi	e the maximum numl	ber of decim	nal
places	to display value	in the outp	ut

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

## 7 Graphic configuration

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

Setting	X
Graphic Configuration Colour Settin	ng   Color Picking
Di 1	
Show Omitted	
Je Show Omreced	Show All the Markers
T Show Value	🔽 Show Epistasis
🔲 Show QTL Position	T Show QTL Position S.I
_ Size	
Space among Chromosomes	300
Effect Size	5
Chromosome Length	6
Chromosome Width	2.5
Zoom in Effect	1
Epistasis Line Width	2

**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.

### 8 Save pictures and reports

### 8.1 Save pictures.

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

Edit( <u>E</u> )	
Save Picture( <u>S</u> )	Ctrl+S
Quick SP(Q)	Ctrl+Q
Save Report( <u>R</u> )	Ctrl+R

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.

### 8.2 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.

#### Part 2 \_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 is 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 3 \_QTL\_heritability (heritabilities of QTL effects)

h^2(a) – The heritability of additive effect.

h<sup>2</sup>(d)– The heritability of dominance effect.

h^2(ae)- The heritability of additive by environment interaction effects.

h^2(de)- The heritability of dominance by environment interaction effects.

#### Part 4 \_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 5 \_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 6** \_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 7 \_superior\_genotype (QTL geneotypes 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.