Users' Manual of QTL IciMapping v3.1

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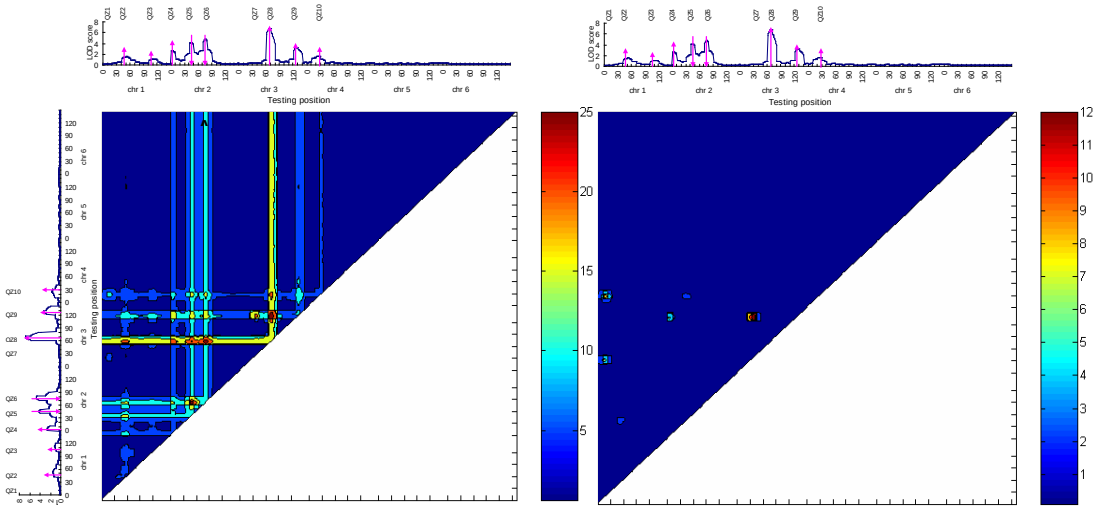

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Chapter 1. Introduction

1.1 Genetic populations in linkage analysis and QTL mapping

Concerning the populations in plants used for genetic linkage analysis and QTL mapping, such as F_2, backcross (BC), doubled haploids (DH), recombination inbred lines (RIL), etc., two categories can be classified: temporary populations and permanent populations. In a temporary population such as F_2 or BC, individuals in the population may segregate after self-pollination. In contrast, in a permanent population such as DH and RIL, each individual in the population is genetically homozygous, and the genetic construction will not change through self-pollination. Thus, in permanent populations the phenotypic value of complex quantitative traits can be measured repeatedly through a replicated experiment design, and the same genotype can be tested under different environments, allowing the study of genotype × environment interaction. Therefore, with permanent populations the random environmental errors can be better controlled and the precision of QTL mapping can be improved. Twenty populations derived from a biparental cross can be handled in QTL IciMapping (Figure 1.1).

Recently, permanent populations consisting of series of chromosome segment substitution (CSS) lines (also called introgression lines) (Figure 1.1) have been used for gene fine mapping. In the idealized case that each CSS line has a single segment from the donor parent, the standard analysis of variance (ANOVA), followed by multiple mean comparison between each line and the background parent, can be readily used to test if the segment in the tested CSS line carries QTLs controlling the trait of interest. Unfortunately, it will take much labor and time to develop a population consisting of idealized CSS lines. Usually in a preliminary CSS population each line carries a few segments from the donor parent. Due to high intensity selection in the process to generate CSS lines, the gene and marker frequencies with CSS lines do not follow the same path as in a standard mapping population such as F_2, BC, DH, or RIL. A likelyhood ratio test based on stepwise regression is impelemented in QTL IciMapping for the non-idealized CSS lines, and is also applicable for idealized CSS lines.

Nested association mapping (NAM) population is derived from a multiple-cross mating design sharing one common parent (Figure 1.1), which may provide high power and high resolution through joint linkage and association analysis, and a broader genetic resource for quantitative trait analysis. NAM populations can also be handled in QTL IciMapping by a joint linkage mapping approach.

By QTL IciMapping, QTL mapping studies can be conducted for the 20 biparental populations, CSS lines and NAM populations (Figure 1.1). Linkage map building is confined in the 20 biparental populations.

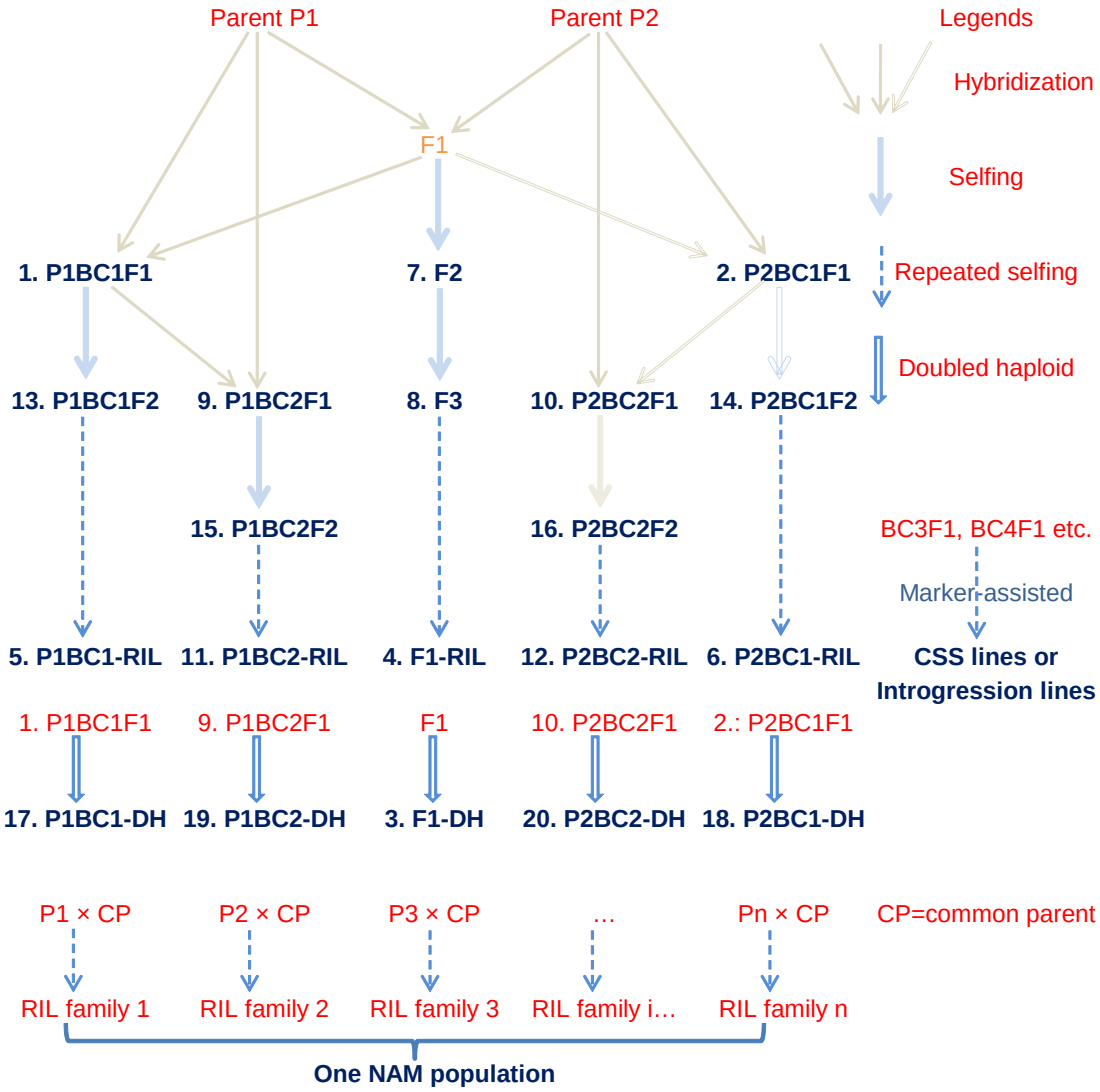


Figure 1.1 Twenty biparental populations that can be used in QTL IciMapping

1.2 Estimation of recombination frequency

We take two populations, i.e., DH and F_2, as examples to illustrate the estimation of recombination frequency. For DH population, the observed number of the four genotypes can be arrayed in matrix notation as,

$$\mathbf{n} = \begin{matrix} \\ BB \\ bb \end{matrix} \begin{matrix} \begin{matrix} AA & aa \end{matrix} \\ \begin{bmatrix} n_{22} & n_{02} \\ n_{20} & n_{00} \end{bmatrix} \end{matrix},$$

where the first and second subscripts of *n* denote genotypes at maker loci A and B,

respectively. The genotype frequencies for marker loci A and B can be arrayed as,

$$\mathbf{F} = \begin{matrix} \\ BB \\ bb \end{matrix} \begin{matrix} \begin{matrix} AA & aa \end{matrix} \\ \begin{bmatrix} \frac{1}{2}(1-r) & \frac{1}{2}r \\ \frac{1}{2}r & \frac{1}{2}(1-r) \end{bmatrix} \end{matrix},$$

where r is the recombination frequency between loci A and B. Using the matrices **n** and **F**, the likelihood function of r given the marker data is,

$$L(r|n) = \frac{n!}{n_{22}!n_{20}!n_{02}!n_{00}!}\left[\frac{1}{2}(1-r)\right]^{n_{22}+n_{00}}\left[\frac{1}{2}r\right]^{n_{20}+n_{02}}.$$

The maximum likelihood estimate (MLE) of the recombination fraction loci A and B can be obtained by differentiating log$L(r|n)$ with respect to r, setting the derivative equal to zero, and solving the resulting function. The MLE of r is,

$$\hat{r} = \frac{n_{20} + n_{02}}{n}.$$

In F_2 population for co-dominance markers, the observed number of the nine genotypes can be arrayed in matrix notation as,

$$\mathbf{n} = \begin{matrix} \\ BB \\ Bb \\ bb \end{matrix} \begin{matrix} \begin{matrix} AA & Aa & aa \end{matrix} \\ \begin{bmatrix} n_{22} & n_{12} & n_{02} \\ n_{21} & n_{11} & n_{01} \\ n_{20} & n_{10} & n_{00} \end{bmatrix} \end{matrix},$$

where the first and second subscripts of n denote genotypes at maker loci A and B, respectively. The genotype frequencies for marker loci A and B can be arrayed as,

$$\mathbf{F} = \begin{bmatrix} \frac{1}{4}(1-r)^2 & \frac{1}{2}r(1-r) & \frac{1}{4}r^2 \\ \frac{1}{2}r(1-r) & \frac{1}{2}\left[(1-r)^2 + r^2\right] & \frac{1}{2}r(1-r) \\ \frac{1}{4}r^2 & \frac{1}{2}r(1-r) & \frac{1}{4}(1-r)^2 \end{bmatrix},$$

where r is the recombination frequency between loci A and B. Using the matrices **n** and

F, the likelihood function of r given the marker data is,

$$L(r|n) = \frac{n!}{n_{22}!n_{12}!\cdots n_{10}!n_{00}!}\left[\frac{1}{4}(1-r)^2\right]^{n_{22}+n_{00}}\left[\frac{1}{2}r(1-r)\right]^{n_{12}+n_{21}+n_{01}+n_{00}}\left[\frac{1}{4}r^2\right]^{n_{02}+n_{20}}\left[\frac{1}{2}(1-r)^2+\frac{1}{2}r^2\right]^{n_{11}}.$$

Although we can differentiate log$L(r|n)$ with respect to r, and solving the function by setting the derivation equal to zero, there is no analytic solution. Since log$L(r|n)$ is a double-differentiable and convex function, we use Newton-Raphson method to maximum $L(r|n)$ directly. In our software, the recombination frequencies in F_2, F_3, $P_1BC_1F_2$, $P_2BC_1F_2$, $P_1BC_2F_2$, and P_2BC_2F2 populations were estimated by Newton-Raphson method, since there is no analytic solution for their maximum likelihood functions. Except these six populations, the recombination frequencies in other 14 populations were estimated in the same way as what we demonstrated for DH population.

1.3 Construction of linkage map

Three steps are invluvled in building a linkage map: Grouping, Ordering and Rippling. All markers are firstly grouped. The grouping in QTL IciMapping can be based on (i) anchored marker information, (ii) a threshold of LOD score, and (iii) a threshold of marker distance. Three ordering algorithms were implemented in QTL IciMapping.

The ordering algorithm of SER is an abbreviation of SERiation. The detailed information of SER can be found in Buetow and Chakravarti (1987) (Am. J. Hum. Genet. 41: 180-188). The ordering algorithm for n loci is as follows. Consider a distance matrix of pairwise recombination values for n loci where r_{ij} is the estimated recombination value between the i^{th} and j^{th} locus in the matrix. For each locus L_i, i = 1, 2, . . ., n (referred to as the reference locus), write locus L_i. Consider the distance between L_i and the other (n - 1) loci. Select the locus (L_j) with the smallest distance from L_i and place it to the right of L_i, i.e., L_i - L_j. For the remaining (n - 2) loci in the row referenced by L_i, the following procedure is repeated: (i) Choose the locus L_k from the remaining unplaced loci in that row with the smallest distance to L_i. (2) Compare the distance of L_k with the two loci currently external in the cluster of placed loci, L_l (the locus on the left side) and L_r (the locus on the right side), i.e., L_l, …, L_r. If $r_{kr} > r_{kl}$, place L_k to the left of the cluster of currently placed loci, i.e., L_k, L_l, …, L_r, or, if $r_{kr} < r_{kl}$, place L_k to the right of the cluster of currently placed loci, i.e., L_l, …, L_r, L_k. The procedure was repeated until all the markers were positioned, therefore providing n orders (one for each marker at the initial position). For each order, the continuity index was calculated continuity index (CI). The best order was considered the one that gave the smallest CI value.

The ordering algorithm of RECORD comes from REcombination Counting and ORDering, proposed by van Os et al. (2005) (Theor. Appl. Genet. 112: 30-40).

Instead of recombination frequency, the pair-wise expected number of recombination events was calculated from genotyping data in a mapping population. Let *n* be the number of loci in ordering, and S_{ij} represent the estimated number of recombination events between the i^{th} and j^{th} locus. The criterion COUNT for a given sequence of loci is calculated by a simple addition of those numbers of recombination events over the proper (adjacent) loci. The core of the ordering algorithm of RECORD for the *n* loci is as follows. First, a sequence is constructed stepwise, starting with a randomly chosen pair of markers, and adding one marker at a time. For each marker to be added the best position is determined (one out of *n*+1 positions if the current sequence has *n* elements). This is a branch-and-bound-like procedure. The order in which markers are added to the sequence is random. Once all markers have been added to the linkage group, thus making a sequence, an additional search for improvement is performed in the following way. A window of a given size is moved along the sequence from head to tail and for every position of this window, the subsequence within the window is inverted, and the resulting COUNT-value calculated. If the reverse order did not offer a lower COUNT-value, the inversion was restored. If a lower COUNT-value was obtained, subsequent steps were done given the new order. This is repeated for windows of increasing size, starting with size two, until the window covers all but one of the loci in the sequence. Every improvement encountered this way is accepted. The whole procedure is repeated until no further improvements are encountered.

The construction of linkage maps has been recognized as a special case of the traveling salesman problem (TSP). We proposed a TSP multi-fragment (MF) heuristic algorithm for ordering of a group of markers.

After ordering, each marker sequence can be rippled as fine tuning. Rippling was done by permuation of a window of six markers and comparison of *m*!/2 resulting maps. Initially, positions 1,…, *m* were permutated, then position 2, …, *m*+1 were permutated, and so on until the whole map was covered. Five rippling criteria are (i) SARF (Sum of Adjacent Recombination Frequencies, $\sum_{i=1}^{m-1} \hat{r}_{M_i M_{i+1}}$, where $\hat{r}$ is the estimation for recombination frequency; Falk, 1989), (ii) SAD (Sum of Adjacent Distances, $\sum_{i=1}^{m-1} \hat{d}_{M_i M_{i+1}}$, where $\hat{d}$ is the estimation for distance), (iii) SALOD (Sum of Adjacent LOD scores, $\sum_{i=1}^{m-1} LOD_{M_i M_{i+1}}$; Weeks and lange, 1987), (iv) COUNT (number of recombination events, $\sum_{i=1}^{m-1} \hat{c}_{M_i M_{i+1}}$; where $\hat{c}$ is the number of recombination events), and (v) LogL (Logrithm Likelihhod of the marker sequence).

1.4 Statistical methods of QTL mapping

Rapid increase in the availability of fine-scale genetic marker maps has led to the intensive use of QTL mapping in the genetic study of quantitative traits. A number of

statistical methods have been developed for QTL detection and effect estimation. From a statistical perspective, methods for QTL mapping are based on three broad classes: regression, maximum likelihood, and Bayesian models. The simplest single marker analysis identifies QTL based on the difference between the mean phenotypic values of different marker groups, but cannot separate the estimates of recombination fraction and QTL effect. Simple interval mapping (SIM) is based on maximum likelihood parameter estimation and provides a likelihood ratio test for QTL position. Regression interval mapping was proposed to approximate maximum likelihood interval mapping to save computation time at one or multiple genomic positions. The major disadvantage of SIM is that the estimates of locations and effects of QTL may be biased when QTL are linked. Composite interval mapping (CIM) combines SIM with multiple marker regression analysis, which controls the effects of QTL on other intervals or chromosomes onto the QTL that is being tested, and thus increases the precision of QTL detection.

It has long been recognized that epistasis or interactions between non-allelic genes plays an important role in the genetic control and evolution of quantitative traits. The pattern of epistasis for a trait can be very complex, and therefore it is difficult to identify the epistatic networks and estimate the epistatic effects. Our knowledge of how interacting genes influence the phenotype of quantitatively inherited traits remains incomplete. Statistical methodology for epistasis mapping is still under development. Some mapping methods based on frequencist statistics, such as interval mapping and regression interval mapping may be extended for mapping epistasis, but the mapping power was low as the background genetic variation was not well controlled. Multiple interval mapping (MIM) fits multiple putative QTL effects and associated epistatic effects simultaneously in one model to facilitate the search, test and estimation of positions, effects and interactions of multiple QTL. However, it requires determining the number of model terms (main effect and epistasis) in the model. As this is usually unknown, various models of different complexities have to be tested. Different MIM model selection methods implemented in the popular software of QTL Cartographer give different, sometimes controversial mapping results, and the nature of the preferred model selection method is not clear.

In some one-dimensional scanning methods for epistasis, either the large mapping populations derived from multiple related inbred-line crosses are required, or the effective dimension of the epistatic effects needs to be specified by users. Assuming that QTL are at marker positions, multiple regression using modified Schwarz Bayesian information criterion has been proposed to map digenic interactive QTL. However, it is not a true QTL mapping method since it cannot estimate the positions and to some extent QTL effects.

The use of Bayesian models in QTL mapping has been widely studied in recent years. Earlier Bayesian models estimated the locations and the effect parameters for a prespecified number of QTL, which is normally unknown before mapping. To solve

this problem, Bayesian methods implemented via the reversible jump Markov chain Monte Carlo (MCMC) algorithm have been proposed. However, Bayesian models have not been widely accepted due to the difficulty and arbitrary in choosing priors, intensive computing requirements, and lack of efficient implementing algorithm and user-friendly software.

1.5 Principle of Inclusive Composite Interval Mapping (ICIM)

Under the assumption of additivity of QTL effects on the phenotype of a trait in interest, the additive effect of a QTL can be completely absorbed by the two flanking marker variables, and the epistatic effect between two QTL can be completely absorbed by the four marker-pair multiplication variables between the two pairs of flanking markers. Based on this property, we proposed a statistical method for QTL mapping, which was called inclusive composite interval mapping (ICIM). Marker variables were considered in a linear model in ICIM for additive mapping, and both marker variables and marker-pair multiplications were simultaneously considered for epistasis mapping. Two steps were included in ICIM. In the first step, stepwise regression was applied to identify the most significant regression variables in both cases but with different probability levels of entering and removing variables. In the second step, a one-dimensional scanning or interval mapping was conducted for mapping additive and a two-dimensional scanning was conducted for mapping digenic epistasis.

In the interval mapping for additive mapping, the phenotypic values were adjusted by all markers retained in the regression equation except the two markers flanking the current interval. The adjusted phenotype in this case contains the position and additive effect information of QTL in the current testing position, and excludes the influence of the QTL located on other interval or other chromosomes. The adjusted observation does not change until the testing position moves into a new interval. Comparably, in the two-dimensional scanning for epistasis mapping, the phenotypic values were adjusted by all variables retained in the regression equation except the two pairs of markers flanking the two current mapping intervals and the four marker-pair multiplications between the two pairs of markers. The adjusted phenotype thus obtained contains the information of QTL in the two testing intervals, which includes two positions and two additive effects of individual QTL, and the interaction between the two QTL. At the same time, the effects of QTL located on other intervals and chromosomes and their epistatic effects have been completely controlled through the introduction of other coefficients. The adjusted observation does not change until either of the two testing positions moves into a new interval.

If dominance effects are included, the regression model in ICIM consists of both marker variables and maker pairs to control additive and dominance effects.

ICIM provides intuitive statistics for testing additive, dominance and epistasis, and can be used for experimental populations derived from two inbred parental lines. The EM

algorithm used in ICIM has a fast convergence speed and is therefore less computing intensive. For additive mapping, ICIM retains all advantages of CIM over interval mapping (IM), and avoids the possible increase of sampling variance and the complicated background marker selection process in CIM. Extensive simulations using different genomes and various genetic models indicate that ICIM has increased detection power, reduced false detection rate and less biased estimates of QTL effects compared to CIM in additive(and dominance) mapping. Extensive simulations also show that ICIM is an efficient method for epistasis mapping, and QTL epistatic networks can be identified no matter whether the two QTL have any additive effects.

1.6 QTL mapping with non-idealized chromosome segment substitution lines

Chromosome segment substitution (CSS) lines have the potential for QTL fine mapping and map-based cloning. But CSS lines with more than one chromosome substitution segment make it impossible to locate QTL on a single chromosome segment through the comparison of the trait performance between one CSS line and the background parent. We present a likelihood ratio test based on stepwise regression (RSTEP-LRT) that can be used for QTL mapping in a population consisting of non-idealized CSS lines. The stepwise regression was used to select the most important segments for the trait of interest, and the likelihood ratio test was used to calculate the LOD score of each chromosome segment. This method is equivalent to the standard *t*-test with idealized CSS lines. To further improve the power of QTL mapping, a method was proposed to decrease multicollinearity among markers (or chromosome segments).

Three steps are needed in the analysis: (1) detecting multicolinearity and deleting redundant markers; (2) performing marker selection using stepwise regression; and (3) conducting likelihood ratio test to declare statistical significance for each marker. Multicollinearity occurs when using regression analysis of trait performance on chromosome segments. One option for removing multicollinearity is to delete redundant markers. In this method, we propose deleting the most correlated markers to decrease the multicollinearity among markers. The decrease in multicollinearity increases the mapping power, but has one disadvantage, i.e., the QTL on deleted markers cannot be identified. But the correlation between a deleted marker and a retained marker showing evidence of QTL can be used as the basis for a conjecture about whether the deleted marker is associated with a QTL.

1.7 Joint Inclusive Compossite Interval Mapping (JICIM)

Nested association mapping (NAM) is a novel genetic mating design that combines the advantages of linkage analysis and association mapping. This design provides

opportunities to study the inheritance of complex traits, but also requires more advanced statistical methods.We proposed a method called joint inclusive composite interval mapping (JICIM), which is an efficient and specialty method for the joint QTL linkage mapping of genetic populations derived from the NAM design.

Chapter 2. Structure of the QTL IciMapping Software

2.1 Development and application environments

In QTL IciMapping, kernel modules for building linkage maps were written by C#, those for QTL mapping was written by Fortran 90/95, and the interface was written by C#. QTL IciMapping runs on Windows XP/Vista/, with .NET Framework 2.0(x86)/3.0/3.5.

2.2 User interface

Figures 2.1 and 2.2 are the interfaces of an open project with name "D:\DemoIciMapping.ipj", when functionalities MAP and BIP are in use. The interface includes Menu Bar, Tool Bar, Status Bar, Message Button, Task List Button, Project Window, Display Window, and Parameter Setting Window. Two display windows can be seen for the MAP functionality (Figure 2.1), one is used to display marker summary information, and the other is used to display the linkage map information. Other functionalities have similar interface as shown in Figure 2.2. The content of an input or output file is demonatrated in Display Window.

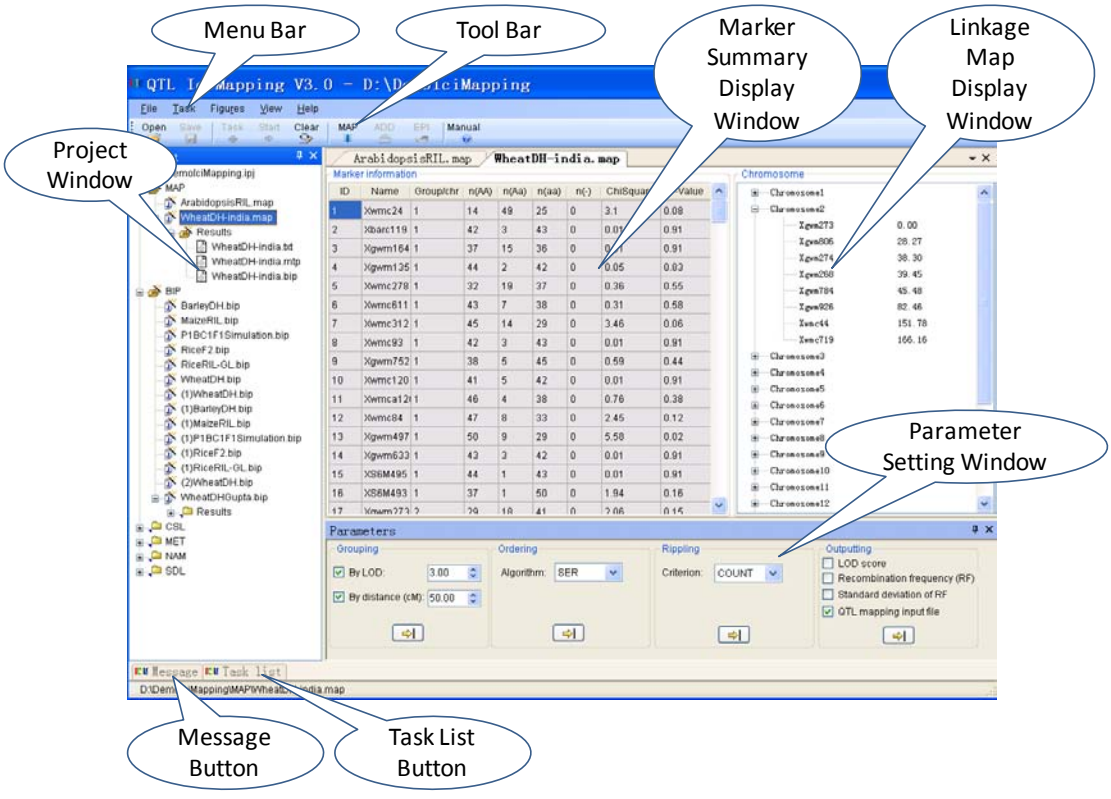


Figure 2.1 The interface of the functionality MAP

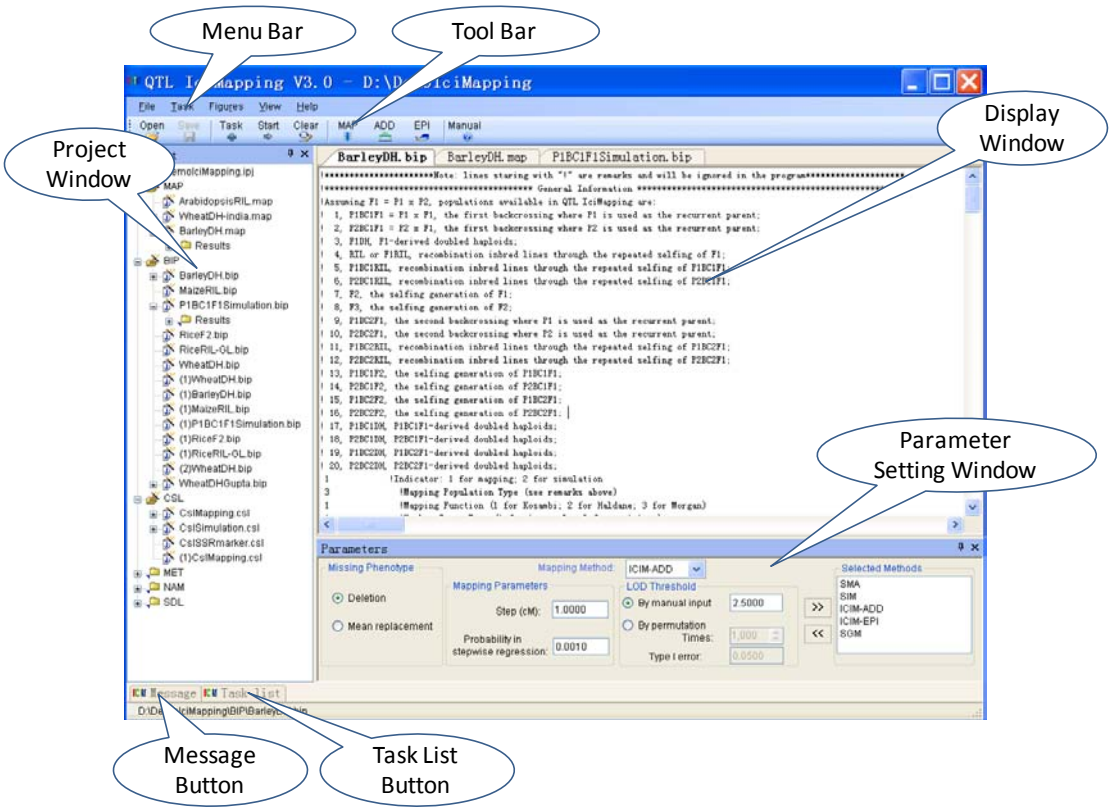


Figure 2.2 The interface of the functionality BIP. Functionalities CSL, MET, NAM and SDL have the similar interface as BIP.

2.3 Functionalities

We provided six major functionalities in QTL IciMapping, i.e. MAP, BIP, CSL, MET, NAM and SDL (Figure 2.3). These functionalities were briefly described as follows.

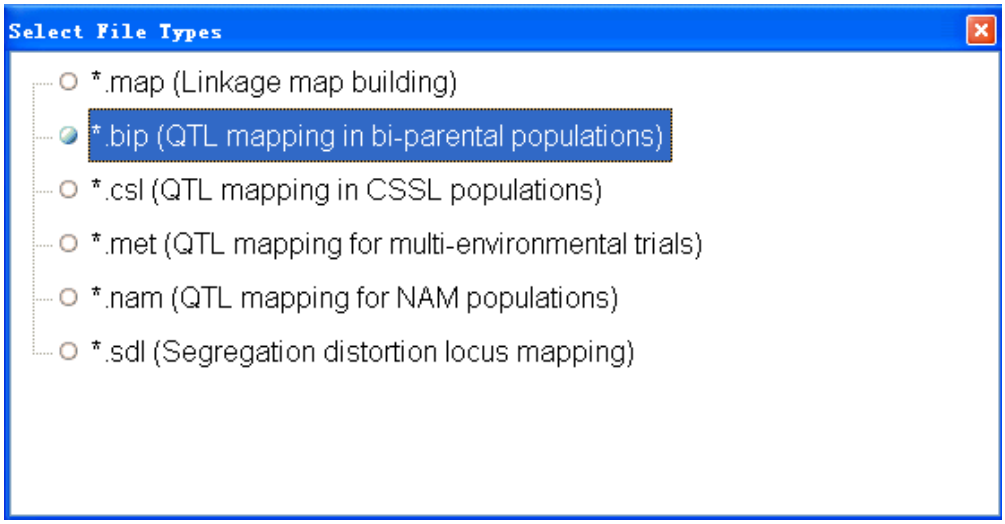


Figure 2.3 The functionality window after selecting the tool bar Open

2.3.1 MAP, construction of genetic linkage maps in biparental populations

There are three steps in building a linkage map: Grouping, Ordering and rippling. Grouping can be based on (i) anchored marker information, (ii) a threshold of LOD score, and (iii) a threshold of marker distance. Three ordering algorithms are (i) SER: SERiation (K. H. Buetow and A. Charavarti. 1987. Am. J. Hum. Genet. 41: 180-188), (ii) RECORD: REcombination Counting and ORDering (H. Van Os. 2005. Theor. Appl. Genet. 112: 30-40), and (iii) MF: Multi-Fragment heuristic algorithm. Five rippling criteria are (i) SARF (Sum of Adjacent Recombination Frequencies), (ii) SAD (Sum of Adjacent Distances), (iii) SALOD (Sum of Adjacent LOD scores), (iv) COUNT (number of recombination events), and (v) LogL (Logarithm Likelihood of the marker sequence).

2.3.2 BIP, mapping of additive and digenic epistasis genes in biparental populations

Five methods are available in BIP, i.e., (i) SMA: Single Marker Analysis (K. Sax. 1923. Genetics 8: 552-560; M. Soller and T. Brody. 1976. Theor. Appl. Genet. 47: 35-39), (ii) IM: the conventional Interval Mapping (E. S. Lander and D. Botstein. 1989. Genetics 121: 185-199), (iii) ICIM-ADD: Inclusive Composite Interval Mapping of ADDitive (and dominant) QTL (H. Li et al. 2007. Genetics 175: 361-374; L. Zhang et al. 2008. Genetics 180: 1177-1190), (iv) ICIM-EPI: Inclusive Composite Interval Mapping of digenic EPIstatic QTL (H. Li et al. 2008. 116: 243-260), and (iv) SGM: Selective Genotyping Mapping (R. L. Lebowitz et al. 1987 Theor. Appl. Genet. 73: 556-562; E. S. Lander and D. Botstein. 1989. Genetics 121: 185-199; Y. Sun et al. 2010. Mol. Breed.).

2.3.3 CSL, mapping of additive and digenic epistasis genes with chromosome segment substitution (CSS) lines

Three methods are available in CSL, i.e., (i) SMA: Single Marker Analysis (K. Sax. 1923. Genetics 8: 552-560), (ii) RSTEP-LRT-ADD: Stepwise regresson based likelihood ratio tests of additive QTL (J. Wang et al. 2006. Genet. Res. 88: 93-104; J. Wang et al. 2007.Theor. Appl. Genet. 115: 87-100), and (iii) RSTEP-LRT-EPI: Stepwise regresson based likelihood ratio tests of digenic epistasis QTL (J. Wang et al. 2006. Genet. Res. 88: 93-104; J. Wang et al. 2007.Theor. Appl. Genet. 115: 87-100).

2.3.4 MET, mapping of additive and digenic epistasis genes from multi-environmental trials

Two methods are available in MET or QTL by E mapping, i.e., (i) ICIM-ADD: Inclusive Composite Interval Mapping of ADDitive (and dominant) QTL (H. Li et al. 2007. Genetics 175: 361-374; L. Zhang et al. 2008. Genetics 180: 1177-1190), and (ii) ICIM-EPI: Inclusive Composite Interval Mapping of digenic EPIstatic QTL (H. Li et al. 2008. 116: 243-260).

2.3.5 NAM, joint inclusive composite interval mapping for NAM populations

Joint inclusive composite interval mapping (JICIM) is available for NAM populations.

2.3.6 SDL, segregation distortion locus mapping

Two methods are available in SDL, i.e., (i) SMA: Single Marker Analysis (K. Sax. 1923. Genetics 8: 552-560; M. Soller and T. Brody. 1976. Theor. Appl. Genet. 47: 35-39), and (ii) IM: the conventional Interval Mapping (E. S. Lander and D. Botstein. 1989. Genetics 121: 185-199).

2.4 Menu bar

- File: open and close project files and input files (Figure 2.4A)
  - New Project: To create a new project
  - Open Project: To open an existing project
  - Save Project: To save the current project
  - Close Project: To close the current project
  - Open File: To include an MAP, BIP, CSL, MET, NAM or SDL file into the current project.
  - Recent Projects: To open a recently used project
  - Exit: To exit the QTL IciMapping

- Task: To manage a batch of QTL mapping jobs (Figure 2.4B)
  - Start QTL mapping: to start the QTL mapping for a batch of BIP files or CSL files in the Task List
  - Stop QTL mapping: to stop the QTL mapping task
  - Add to task list: Add a QTL mapping job to the Task List
  - Refresh task list: To refresh the Task List

- Figures: To view results in graphs (Figure 2.4C)
  - Linkage map: To view the linkage maps
  - ICIM for additive mapping: To view the results from ICIM additive mapping
  - ICIM for epistatic mapping: To view the results from ICIM digenic epistatic mapping
  - Simple interval mapping: To view the results from the simple interval mapping
  - Single marker analysis: To view the results from the single marker analysis mapping
  - Selective genotyping: To view the results from the selective genotyping mapping
  - RSTEP-LRT-ADD: To view the results from the stepwise regression based likelihood ratio test for additive QTL with chromosome segment substitution lines

- SMA for CSS lines: To view the results from the single marker analysis with chromosome segment substitution lines

- View: To manage the interface windows (Figure 2.4D)
  - Tool Bar: To open or close Tool Bar
  - Status Bar : To open or close Status Bar
  - Project: To open or close Project Window
  - Parameters: To open or close Parameter Setting Window
  - Message: To open or close Message Window
  - Task List: To open or close Task List Window

- Help: To access help information (Figure 2.4E)
  - Manual: To view the Users' Manual of QTL IciMapping
  - Update: To view the update information of QTL IciMapping
  - About: To view the version information of QTL IciMapping

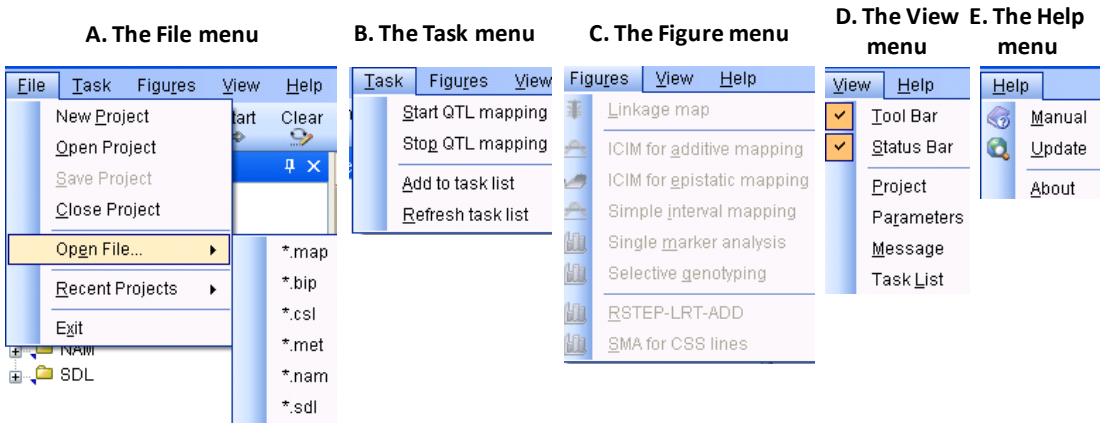


Figure 2.4 Menu bars in QTL IciMapping

2.5 Tool bar

Tool bar provides short cut to the software functionality (Figure 2.5).

- 

: A dialogue window will appear for the users to choose the functionality (Figure 2.2). This tool bar is equivalent to Open Files in the File menu (Figure 2.3A).

- 

: To save the project

- 

: To add a job to the task list

- 

: To start the job in the task list; To start the job in the current parameter window if the task list is empty

- 

: To clear the job and results in the current parameter window

- 

: To draw the linkage map for the current input file. This tool bar is equivalent to Linkage Map in the Figures menu (Figure 2.3C).

- 

: To draw figures from the ICIM additive QTL mapping. This tool bar is equivalent to ICIM for additive mapping in the Figures menu (Figure 2.3C).

- 

: To draw figures from the ICIM epistatic QTL mapping. This tool bar is equivalent to ICIM for epistatic mapping in the Figures menu (Figure 2.3C).

- 

: To view the Users' Manual. This tool bar is equivalent to Manual in the Help menu (Figure 2.3E).

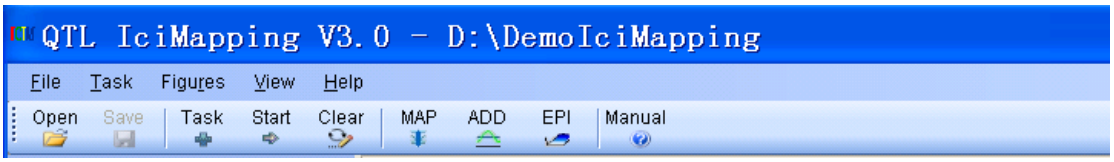


Figure 2.5 Tool bars in QTL IciMapping

2.6 The project concept in QTL IciMapping

QTL IciMapping is project-based software. After you open the software, the first thing to do is to build a new project or open an existing project. The use of project will assure that all operations and results will be properly saved when QTL IciMapping is closed. When the project is open next time, all operations and results previousely done can be recovered. In project "D:\DemoIciMapping" in Figure 2.1, all functionalities were used. Six nodes in the Project Window represent the six used functionalities. Fewer nodes will be displayed if less functionality is used. Contents in the six nodes shown are briefly described as follows.

- The MAP node: All input files for building linkage maps and output files are displayed under this node. Excel files will be converted to the *.MAP format.
- The BIP node: All input files for QTL mapping in biparental populations and output files are displayed under this node. Excel files will be converted to the *.BIP format.

- The CSL node: All input files for QTL mapping in chromosome segment substitution line populations and output files are displayed under this node. Excel files will be converted to the *.CSL format.
- The MET node: All input files for QTL by E analysis in biparental populations and output files are displayed under this node. Excel files will be converted to the *.MET format.
- The NAM node: All input files for QTL mapping in NAM populations and output files are displayed under this node. Excel files will be converted to the *.NAM format.
- The SDL node: All input files for segregation distortion locu mapping in biparental populations and output files are displayed under this node. Excel files will be converted to the *.SDL format.

2.6.1 Start from a new project

To click New Project in the File menu (Figure 2.4A) to build a new QTL IciMapping project. The users will be asked to assign a name to the new project and specify a path to store the project (Figure 2.6).

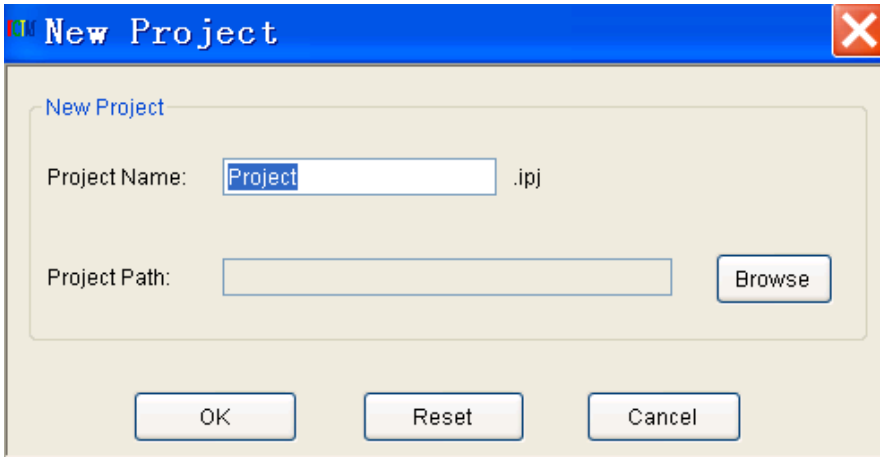


Figure 2.6 The New Project Window

2.6.2 Open an existing project

To click Open Project in the File menu (Figure 2.4A) to open an existing QTL IciMapping project (Figure 2.7). An existing project can also be open through the selection in the Recent Projects list in the File menu (Figure 2.8).

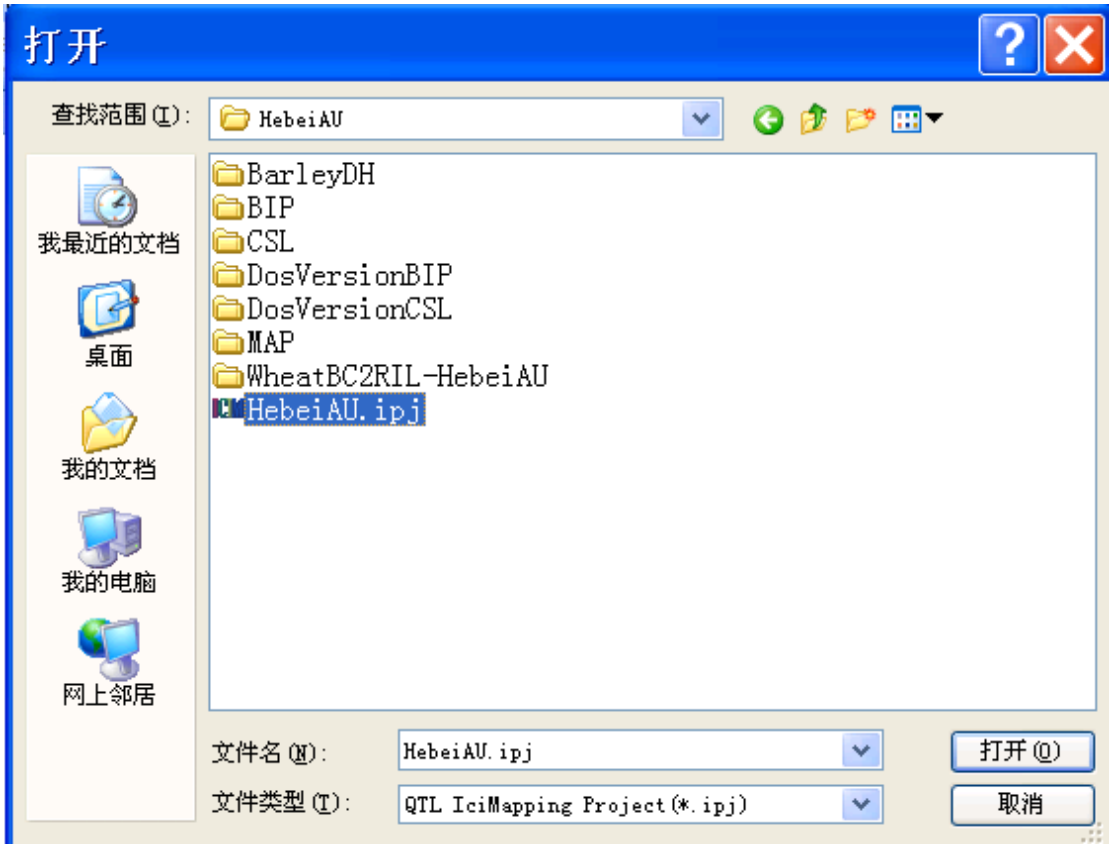


Figure 2.7 Open an existing QTL IciMapping project

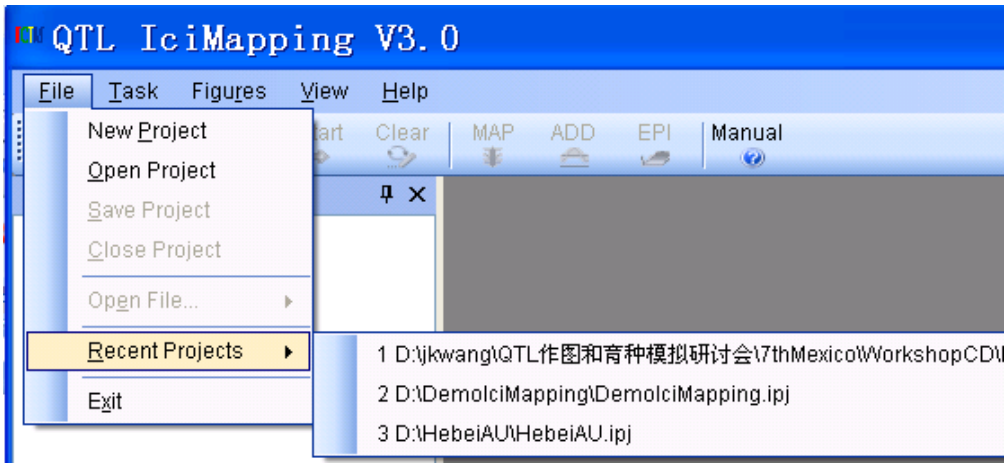


Figure 2.8 Open a recently used QTL IciMapping project

2.6.3 Manage the Project Window

Short-cut menus are provided to manage the project and contents in the project (Figure 2.9).

For the project (Figure 1.9A),

- Open file…: same operation as the tool bar 

- Save: same operation as the tool bar 

- Close: same operation as Close Project in the File menu
- Refresh: refresh contents of the project

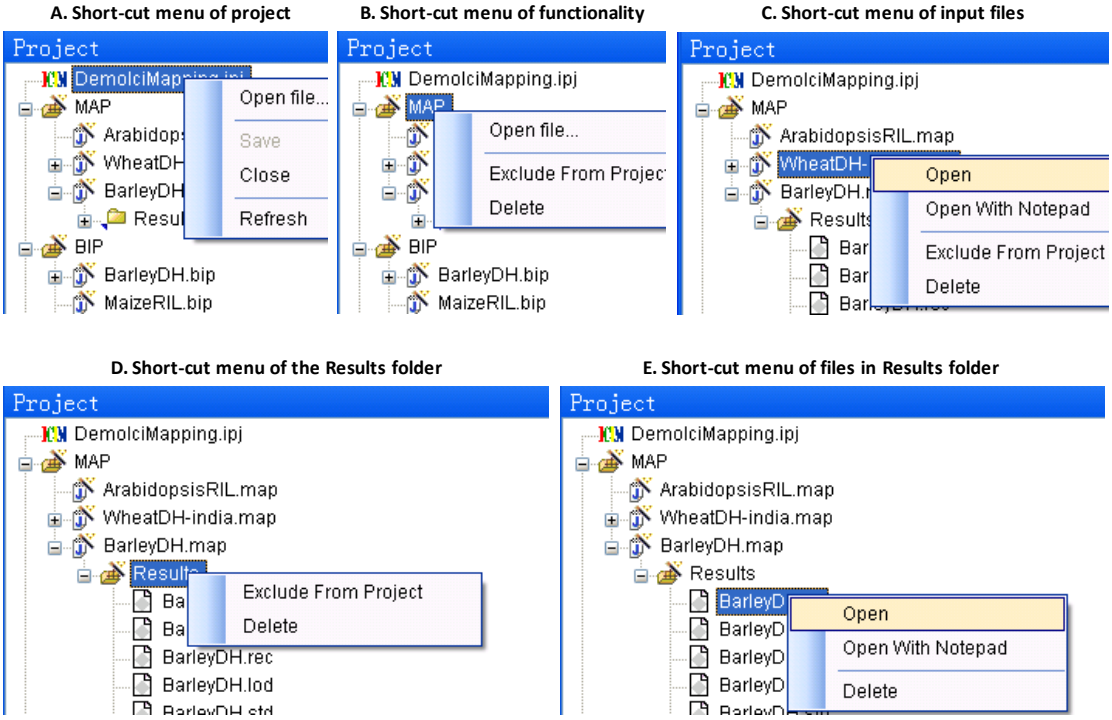


Figure 2.9 Short-cut menus by right clicking the mouse in the Project Window

For a functionality (Figure 1.9B),

- Open file…: open an input file for the current fucntionality
- Exclude From Project: exclude the functionality from the project, but all results from this functionality will not be deleted.
- Delete: exclude the functionality from the project, and all results from this functionality will be deleted.

For an input file (Figure 1.9C),

- Open: open the input file in the Display Window.
- Open With Notepad: open the input file with Notepad.
- Exclude From Project: exclude the iuput file from the project, but all results from this file will not be deleted.
- Delete: exclude the iuput file from the project, and all results from this file will not be deleted.

For the Results folder (Figure 1.9D),

- Exclude From Project: exclude the iuput file from the project, but all results from this file will not be deleted.
- Delete: exclude the iuput file from the project, and all results from this file will not be deleted.

For an output file in the Results folder (Figure 1.9E),

- Open: open the output file in the Display Window.
- Open With Notepad: open the output file with Notepad.
- Delete: to delete the output file from the Results folder.

2.6.4 Manage the Display Window

To click the file name in Figure 2.10A to see the file in the Display Window. Short-cut menus are provided to manage the project and contents in the project (Figure 2.9).

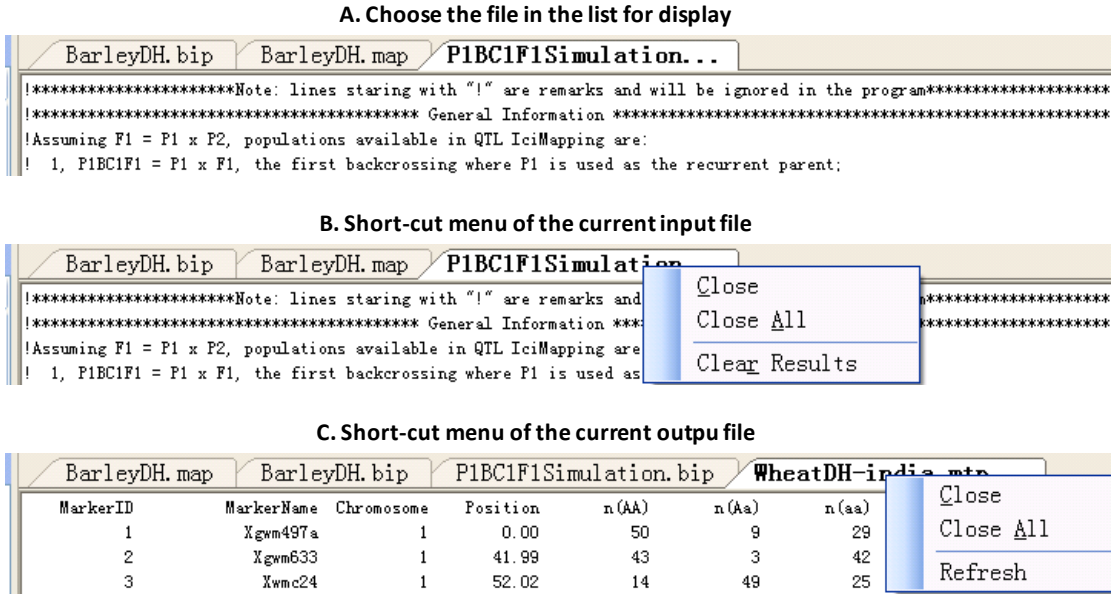


Figure 2.10 Short-cut menus by right clicking the mouse in the Project Window

For an input file, i.e., file with extension name *.map, *.bip, *.csl, *.met, *.nam, or *.sdl (Figure 2.10B),

- Close: close the input file from the Display Window. The file can still be seen in the Project Window.
- Close All: close all input and output files from the Display Window. These files can still be seen in the Project Window.

- Clear Results: same operation as the tool bar 

For an output file (Figure 2.10B),

- Close: close the input file from the Display Window. The file can still be seen in the Project Window.
- Close All: close all input and output files from the Display Window. These files can still be seen in the Project Window.
- Refresh: re-load the output file to the Display Window

2.7 Major folders and files included in the software

The following folders and files will be created in the root directory of QTL IciMapping after the successful installment of the software.

- Example folder: Contain the example input files for functionalities MAP, BIP, CSL, MET, NAM and SDL.
  - MAP folder: contain the example input files for MAP
    - *.map: a pure text format
    - *.xls: the EXCEL 2003 format
    - *.xlsx: the EXCEL 2007 format
  - BIP folder: contain the example input files for BIP
    - *.map: a pure text format
    - *.xls: the EXCEL 2003 format
    - *.xlsx: the EXCEL 2007 format
  - CSL folder: contain the example input files for CSL
    - *.map: a pure text format
    - *.xls: the EXCEL 2003 format
    - *.xlsx: the EXCEL 2007 format
  - MET folder: contain the example input files for MET
    - *.met: a pure text format
    - *.xls: the EXCEL 2003 format
    - *.xlsx: the EXCEL 2007 format
  - NAM folder: contain the example input files for NAM
    - *.nam: a pure text format
    - *.xls: the EXCEL 2003 format
    - *.xlsx: the EXCEL 2007 format
  - SDL folder: contain the example input files for SDL
    - *.sdl: a pure text format
    - *.xls: the EXCEL 2003 format
    - *.xlsx: the EXCEL 2007 format

- ICIM_kernel folder: Contain the computing modules written in Fortron 90/95. Files

contained in this folder are:

- BIP.exe: computing module of BIP
- BIP_mapping.par: parameters called by BIP mapping function
- BIP_simulation.par: parameters called by BIP simulation function
- CSL.exe: computing module of CSL
- CSL_mapping.par: parameters called by CSL mapping function
- CSL_simulation.par: parameters called by CSL simulation function
- MET.exe: computing module of MET
- CSL_mapping.par: parameters called by MET mapping function
- NAM.exe: computing module of NAM
- NAM_mapping.par: parameters called by NAM mapping function
- SDL.exe: computing module of SDL
- SDL_mapping.par: parameters called by SDL mapping function
- FindQTL.mio: an text file to specify the name of file to be run by BIP, CSL, MET, NAM, or SDL

- DockPanel.config: Config file of the software
- QTL IciMapping.exe: the main program of QTL IciMapping
- ICSharpCode.SharpZipLib.dll: An DLL to read zipped files
- WeifenLuo.WinFormsUI.Docking.dll: A DLL to setup the windows
- ZedGraph.dll: A DLL to make graphs
- ReadDataBIP.dll: A DLL to verify BIP input files
- ReadDataCSL.dll: A DLL to verify CSL input files
- DFORRT.DLL: A system DLL
- DFORRTD.DLL: A system DLL
- FICIM.dll: A system DLL
- MSVCRTD.DLL: A system DLL
- Readme.txt: A brief introduction of the software

2.8 Miscellaneous

2.8.1 See the task list

One may want to set a batch of jobs and then run them together. Task List provided

such an option. Under functionalities BIP, CSL, MET, NAM, and SDL, to click the tool bar 

to add a specified job to the Task List, to click the tool bar 

to run all jobs in the task list (Figure 2.11).

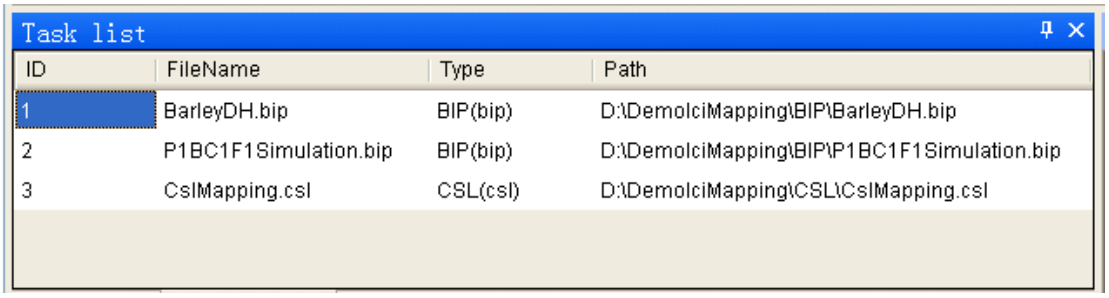


Figure 2.11 Check the Task List

2.8.2 See the running message

Useful message during uploading an input file or running a job can be found in the Message Window (Figure 1.12). When some errors are met during uploading an input file, one can find where the errore are located from the Message Window. When running some big job, for example the epistatic mapping with a small step, one can check the Message Window to see the progress.

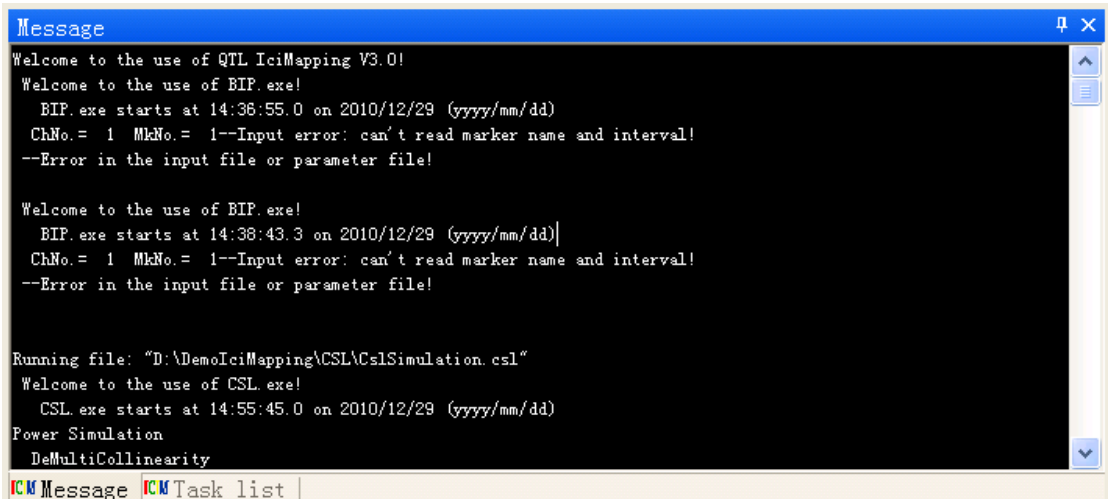


Figure 2.12 Check the Message Window

Chapter 3. Construction of Genetic Linkage Maps (MAP)

3.1. Linkage map input file (*.map or EXCEL)

3.1.1 General information of the mapping population

Five parameters were used for the general information describing the data for linkage map construction (Table 3.1).

- Population Type: describe the type of the population. At present, QTL IciMapping can conduct linkage map construction for twenty populations derived from two parental lines (Figure 1.1). Assuming F1 = P1 x P2, the 20 biparental populations are:
  - 1. P1BC1F1: the backcross population where the first parent (P1) is used as the recurrent.
  - 2. P2BC1F1: the backcross population where the second parent (P2) is used as the recurrent.
  - 3. F1DH: doubled haploids derived from F1.
  - 4. RIL: recombination inbred lines derived from repeated selfing since F1 generation.
  - 5. P1BC1RIL: recombination inbred lines derived from the backcross population where the first parent is used as the recurrent.
  - 6. P2BC1RIL: recombination inbred lines derived from the backcross population where the second parent is used as the recurrent.
  - 7. F2: the selfing generation of F1.
  - 8. F3: the selfing generation of F2.
  - 9. P1BC2F1: the second backcrossing where P1 is used as the recurrent parent.
  - 10. P2BC2F1: the second backcrossing where P2 is used as the recurrent parent.
  - 11. P1BC2RIL: recombination inred lines through the repeated selfing of P1BC2F1.
  - 12. P2BC2RIL: recombination inred lines through the repeated selfing of P2BC2F1.
  - 13. P1BC1F2: the selfing generation of P1BC1F1.
  - 14. P2BC1F2: the selfing generation of P2BC1F1.
  - 15. P1BC2F2: the selfing generation of P1BC2F1.
  - 16. P2BC2F2: the selfing generation of P2BC2F1.
  - 17. P1BC1DH: P1BC1F1-derived doubled haploids.
  - 18. P2BC1DH: P2BC1F1-derived doubled haploids.
  - 19. P1BC2DH: P1BC2F1-derived doubled haploids.
  - 20. P2BC2DH: P2BC2F1-derived doubled haploids.

- Mapping Function: specify the mapping function which will be used to transfer recombination frequency to mapping distance in linkage map construction.

- 1 for Kosombi mapping function.
- 2 for Haldane mapping function.
- 3 for Morgan mapping function.

- Marker Space Type: specify whether the markers on a chromosome (or linkage group) are defined by positions or marker intervals.
  - 1 for intervals, i.e. the number behind a marker is the distance of the marker to its next marker. 0 is normally given for the last marker on a chromosome or a linkage group.
  - 2 for positions, i.e. the number behind each marker is the position of the marker on the chromosome or the linkage group.

- Number of markers: number of markers that need to grouped and ordered into linkage map.

- Population Size: number of individuals in the population.

Table 3.1 General information in a linkage map input file

```
!***Note: lines staring with "!" are remarks and will be ignored in the program*****
!********************General Information ********************************
!Assuming F1 = P1 x P2, populations available in QTL IciMapping are:
!  1, P1BC1F1 = P1 x F1, the first backcrossing where P1 is used as the recurrent parent;
!  2, P2BC1F1 = P2 x F1, the first backcrossing where P2 is used as the recurrent parent;
!  3, F1DH, F1-derived doubled haploids;
!  4, RIL or F1RIL, recombination inbred lines through the repeated selfing of F1;
!  5, P1BC1RIL, recombination inbred lines through the repeated selfing of P1BC1F1;
!  6, P2BC1RIL, recombination inbred lines through the repeated selfing of P2BC1F1;
!  7, F2, the selfing generation of F1;
!  8, F3, the selfing generation of F2;
!  9, P1BC2F1, the second backcrossing where P1 is used as the recurrent parent;
! 10, P2BC2F1, the second backcrossing where P2 is used as the recurrent parent;
! 11, P1BC2RIL, recombination inbred lines through the repeated selfing of P1BC2F1;
! 12, P2BC2RIL, recombination inbred lines through the repeated selfing of P2BC2F1;
! 13, P1BC1F2, the selfing generation of P1BC1F1;
! 14, P2BC1F2, the selfing generation of P2BC1F1;
! 15, P1BC2F2, the selfing generation of P1BC2F1;
! 16, P2BC2F2, the selfing generation of P2BC2F1;
! 17, P1BC1DH, P1BC1F1-derived doubled haploids;
! 18, P2BC1DH, P2BC1F1-derived doubled haploids;
! 19, P1BC2DH, P1BC2F1-derived doubled haploids;
! 20, P2BC2DH, P2BC2F1-derived doubled haploids;
 3       !Mapping Population Type (see remarks above)
 1       !Mapping Function (1 for Kosambi; 2 for Haldane; 3 for Morgan)
 2       !Marker Space Type (1 for intervals; 2 for positions)
 127         !Number of Markers
 145     !Population size of the mapping population
```

3.1.2 Marker type information

In QTL IciMapping, 2 was used to represent the marker type of the first parent (P1), 0 for the second parent (P2), 1 for the F1 marker type, and -1 for any missing markers. The number of marker types behind each marker name has to be exact the population size. Each marker type is separated by space or TAB keys. Each marker can occupy one line, or multiple lines.

Table 3.2 Marker type information in a linkage map input file (incomplete).

```
!************************ Marker Types **********************************
!Marker type: 2 for P1; 1 for F1; 0 for P2; -1 for missing markers
```

```
Act8A       0   2   -1  2   0   2   2   0   0   2   2   2   0   2   2   0   0
    0   2   2   0   0   2   0   0   0   2   0   2   2   0   0   2   0   2   0
    2   0   0   0   0   2   0   0   2   0   0   2   2   0   2   2   0   2   2
    2   0   2   0   2   2   2   0   2   0   2   2   2   2   0   2   2   0   0
    0   0   2   2   2   0   2   2   2   2   0   0   0   2   2   2   0   2   2
    2   0   2   2   0   0   2   2   2   0   0   2   0   0   2   0   0   2   2
    2   0   2   0   2   0   2   0   0   0   0   0   2   2   0   2   2   0   2
    0   0   0   0   0   0   2   0   2   0   0   2   2   2
OP06        0   2   2   2   0   2   2   0   0   2   2   2   0   2   2   0   0
    2   2   2   0   0   2   0   0   0   0   0   2   2   0   0   2   2   0   0
    2   0   0   0   2   0   0   0   2   0   0   2   2   0   2   2   0   2   -1
    2   0   2   0   2   2   2   2   2   0   2   2   2   2   2   2   2   0   0
    0   0   2   0   2   0   2   2   2   2   -1  0   0   2   2   2   0   2   0
    2   0   2   0   0   0   2   2   2   0   0   -1  0   0   2   0   0   2   2
    2   0   2   0   2   0   0   -1  0   0   2   0   2   2   0   0   2   0   2
    0   0   0   2   0   0   2   0   2   0   0   2   2   2
aHor2       0   2   2   2   0   2   2   0   0   2   2   2   0   2   2   0   0
    2   2   2   0   0   2   0   -1  2   2   2   2   -1  -1  0   0   0   2   -1
    2   0   0   0   2   0   0   0   2   0   0   2   2   0   2   2   0   2   2
    2   0   2   0   2   2   2   2   -1  2   2   2   2   2   2   2   2   0   0
    0   0   2   0   2   0   2   2   -1  2   0   0   0   2   2   2   0   2   0
    2   -1  2   0   0   0   2   2   2   0   0   2   0   0   2   0   0   2   2
    2   0   2   0   2   0   0   0   0   0   2   0   2   2   -1  2   2   0   2
    0   0   0   0   2   -1  2   0   2   0   0   2   2   2
MWG943      2   2   2   2   2   2   0   0   0   0   2   0   2   0   2   2   0
    2   2   0   0   2   2   2   -1  0   0   0   2   2   2   2   0   2   2   0
    2   2   2   0   0   2   0   2   0   0   0   -1  0   0   0   2   -1  -1  2
    2   -1  0   2   0   2   2   0   0   2   2   2   2   2   2   2   0   0   2
    2   0   0   0   0   0   2   2   2   2   0   0   0   2   2   0   2   2   0
    2   0   2   2   0   2   -1  2   2   -1  0   0   0   0   2   0   2   -1  2
    2   2   0   2   0   0   0   2   0   0   2   0   0   2   2   0   2   2
    2   2   2   0   0   2   2   0   2   0   0   0   2   2
ABG464      2   -1  2   2   2   2   0   0   0   0   2   0   2   0   2   2   0
    2   2   0   0   2   2   0   2   2   0   0   2   2   2   2   0   2   2   0
    2   2   2   0   0   2   0   2   0   0   0   0   -1  0   0   2   -1  0   2
    2   2   2   2   0   0   2   0   0   2   0   0   2   2   0   2   0   0   2
    2   0   0   0   0   0   2   2   2   2   0   -1  0   2   2   0   2   2   0
    0   0   2   2   2   2   2   2   2   0   0   0   2   0   0   0   2   2   2
    2   2   0   -1  2   2   2   0   0   0   2   0   0   2   2   0   0   2   2
    2   2   2   0   2   2   2   0   0   2   0   0   2   2
Dor3        2   0   2   0   2   2   2   0   0   0   0   2   2   2   2   2   2
    2   2   0   0   2   2   0   0   2   0   0   2   2   2   0   0   2   2   2
    2   2   2   2   0   2   0   2   2   0   0   0   0   0   0   0   0   0   0
    2   0   2   0   0   0   0   0   -1  0   0   0   2   2   0   2   0   -1  2
    2   0   0   0   0   0   2   -1  2   2   0   0   0   2   2   2   0   2   0
    0   0   2   2   2   2   2   0   2   0   0   0   2   0   0   0   2   2   2
    2   -1  0   2   2   2   2   0   0   0   2   2   0   2   2   0   0   0   2
    2   2   0   0   2   2   2   0   0   2   2   2   2   2
iPgd2       2   0   2   0   2   2   2   0   0   0   0   2   2   2   2   2   2
    2   2   0   0   2   2   0   0   2   0   2   2   2   2   0   0   0   2   2
    2   2   2   2   0   2   0   2   2   0   0   0   0   0   0   0   0   0   0
    2   0   2   0   0   0   0   0   0   0   0   0   2   2   0   2   0   0   2
    2   0   0   0   0   0   2   2   2   2   0   0   0   2   2   2   0   2   0
    0   0   2   2   2   0   2   0   2   0   0   0   2   0   0   0   0   2   2
    2   2   0   2   2   2   2   0   0   0   2   2   0   2   2   2   0   0   2
    2   2   0   0   2   2   2   0   0   2   2   2   2   2
cMWG733A    2   0   2   0   2   2   2   2   2   2   0   2   2   2   0   2   2
    2   2   2   0   2   2   0   0   2   0   2   2   2   2   0   0   0   2   2
    2   2   2   2   0   2   0   2   2   0   0   0   0   0   0   0   0   2   0
    2   0   2   0   0   0   0   0   0   0   0   0   2   2   0   2   0   2   2
    2   0   -1  0   0   0   2   2   2   2   0   0   0   2   0   2   -1  2   0
    0   0   2   2   2   0   2   0   2   0   0   0   2   0   0   0   0   2   0
    2   2   0   2   2   2   2   0   0   0   2   2   0   2   2   2   0   0   2
    2   2   0   0   0   2   2   0   0   2   2   2   2   2
AtpbA       0   0   2   0   2   2   2   2   2   2   0   2   2   2   0   2   2
    2   2   2   0   2   2   0   0   2   0   0   2   0   2   0   0   0   2   2
    2   2   2   2   0   2   0   2   2   0   0   0   0   0   0   0   0   2   0
    2   -1  -1  0   0   0   0   0   0   0   0   0   0   2   2   0   2   0   2   2
    2   0   0   0   0   0   2   2   2   2   0   0   0   2   0   2   0   2   2
    0   0   2   2   2   0   2   0   2   0   0   0   2   0   0   0   0   2   0
    2   2   0   2   2   2   2   0   0   0   2   2   2   2   2   2   0   0   2
    2   2   0   0   0   2   2   0   0   2   2   2   2   2
drun8       0   0   2   0   2   2   0   2   2   2   0   2   2   2   0   2   2
    2   2   2   0   2   2   0   0   0   0   2   0   2   0   0   0   0   2   2
    2   2   2   2   0   2   2   2   2   2   0   0   0   0   0   0   0   2   0
    2   0   0   0   0   0   0   0   0   0   2   0   2   2   0   2   0   2   2
```

```
    2   0   0   0   2   0   2   2   0   2   0   0   2   2   0   2   0   2   2
    0   0   0   2   2   0   2   0   0   0   2   0   2   2   0   0   0   2   0
    0   2   0   2   0   2   0   0   0   0   0   2   2   2   2   2   2   0   2
    2   2   0   0   0   2   2   2   0   2   2   2   2   2
ABC261      0   0   2   0   2   2   2   2   2   2   0   2   2   2   0   2   2
    2   2   2   0   2   2   0   0   0   0   0   2   0   2   0   0  -1   0   2
    2   2   2   2   0   2   2   2   2   2   0   0   0   0   0   0   0   2   0
    2   0   0   0   0   0   0   0   0   0   2   0   2   2   0   2   0   2   2
    2   0   0   0   2   2   2   2   0   2   0   0   0   2   0   2   0   2   2
    0   0   0   2   2   0   2   2   0   0   0   0   2   2   0   0   0   2   0
    0   2   0   2   0   2   0   0   0   0   0   2   2   2   2   2   0   0   2
    2   2   0   0   0   2   2   0   0   2   2   2   2   2
ABG710B     0   0   2   0   0   0   2   2   2   2   0   2   2   2   0   2   2
    2   2   2   0   2   0   0   0   0   0   0   2   0   2   0   0   0   0   2
    0   2   2   2   0   2   2   2   2   2   0   0   0   2   0   0   0   2   0
    2   0   0   0   0   0   0   0   0   0   2   0   2   2   0   2   0   2   2
    2   0   0   2   2   2   2   2   0   2   2   0   2   2   0   2   0   2   2
    2   0   0  -1   2   0   2   2   0   0   0   0   2   2   0   0   0   2   0
    0   2   0  -1   0   2   0   0   0   0   0   2   2   2   2   2   0   0   2
    2   2   0   0   0   2   2   0   0   2   0   2   2   2
Aga7        0   0   2   0   0   0   2   2   2   2   0   2   2   2   0   2   2
    2   2   2   0   2   0   0   0   0   0   0   2   0   0   0   0   0   0   2
    0   2   2   2   0   2   2   2   0   2   0   0   0   2   0   0   0   2   0
    2   0   0   0   0   0   0   0   0   0   2   0   2   2   0   2   2   2   2
    0   0   0   2   2   2   2   2   0   2   2   0   2   2   0   2   0   2   2
    2   0   0   0   2   0   2   2   0   0   0   0   2   2   2   0   0   2   0
    0   2   0   2   0   2   0   0   0   0   0   0   2   2   2   2   2   0   0   2
    2   2   0   0   0   2   2   0   0   2   0   2   2   2
MWG912      0   0   2  -1   0   0   2   2   2   2   0   2   2   2   0   2   2
    2   2   2   0   2   0   2   0   0   0   0   2   0   0   0   0   0   2   2
    0   2   0   2   0   2   2   2   0   2   0   0   0   2   0   0   0   2   0
    2   0   0   0   0   0   0   2  -1   2   2   0   2   2   0   2   2  -1   2
    0   0   0   2   2   2   2   2   0   2   2   0   2   2   0   2   0   2   2
    2   0   0   0   2   0   2   2   0   0   2   0   2   2   2   0   0   2   0
    0  -1   0   2   0   2   0   0   0   0   0   2   2   2   2   2   0   0   2
    2   2   0   0   0   0   2   0   0   0   0   2   2   2
…
```

3.1.3 Marker anchoring information

Anchoring information was given in the order of markers defined in Table 3.2. Each marker name was followed by the anchored group ID correspondingly (Table 3.3). If this marker were not anchored, the anchor ID should be fixed as 0. The marker name in this part has to be the same as that specified in Table 3.2.

Table 3.3 Marker anchoring information (incomplete).

```
!*************************** Information for Chromosomes and Markers ***********
Act8A         1
OP06          0
aHor2         0
MWG943        0
ABG464        0
Dor3          0
iPgd2         0
cMWG733A      0
AtpbA         0
drun8         0
ABC261        0
ABG710B       0
Aga7          0
MWG912        1
```

3.1.4 Linkage map input file in EXCEL (*.xls or *.xlsx)

The input data for linkage map construction in biparental populations can also be defined in an Excel file with the extension name ‘xls’ or ‘xlsx’. The file should be

composed of three sheets: ‘GeneralInfo’ (similar to Table 3.1), ‘Genotype’ (similar to Table 3.2), and ‘Anchor’ (similar to Table 3.3).

3.2 Summary of marker data

The MAP functionality can be initiated by (1) opening input files, or (2) double clicking MAP files listed in the project window, or (3) clicking one MAP file in the display window (Figure 2.1). When functionality MAP is activated, the window at the bottom is for parameter setting. The Display Window is split into two windows: the one on the left shows marker summary information, and the one on the right shows resuts from Grouping, Ordering and Rippling.

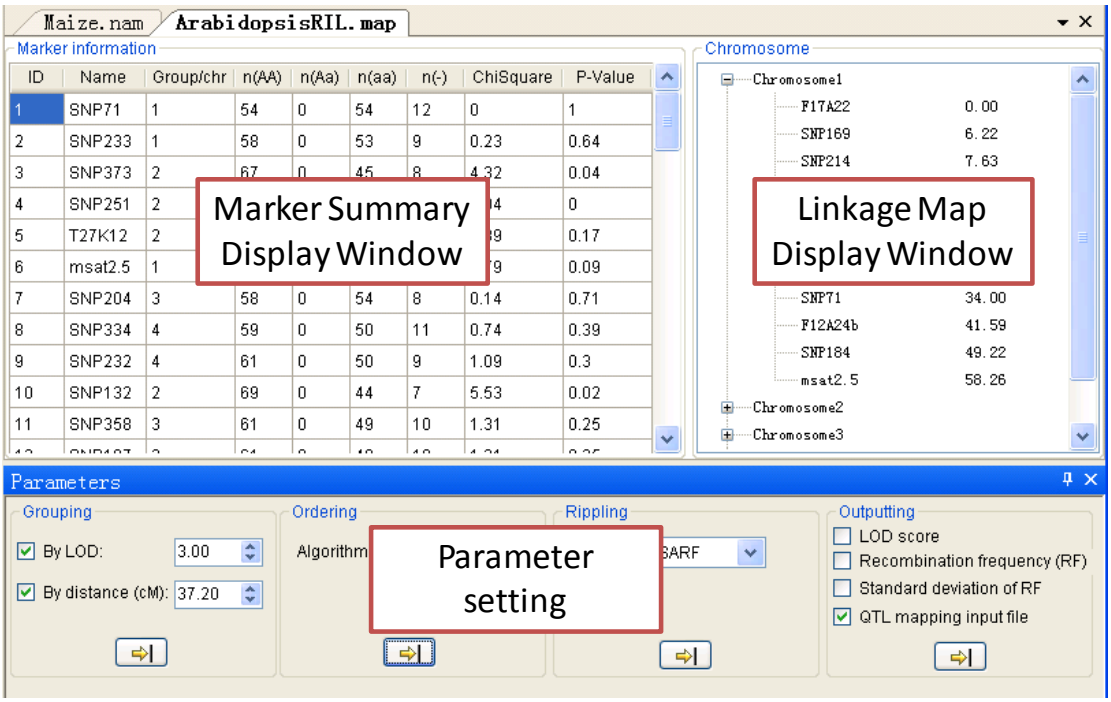


Figure 3.1 The three windows in the MAP functionality

Rows of anchored markers are colored in Marker Summary Display Window. The summary statistics for markers can be rearranged by clicking any column. The meaning of each column is described as follows.

- ID: marker ID in the order with that in the input file.
- Name: marker name in the order with that in the input file.
- Group/chr: for the anchoring group number consistent with that is the input file. If marker was not anchored or have been deleted, this value should be 0.
- n(AA): the number of genotypes same as the first parent (P1), which denoted as 2 in the input file.
- n(Aa): the number of genotypes same as F1, which denoted as 1 in the input file.
- n(aa): the number of genotypes same as the second parent (P2), which denoted

as 0 in the input file.

- n(-): the number of missing genotypes, which denoted as -1 in the input file.
- ChiSquare: χ^2-test statistics testing for segregation distortion of markers.
- P-Value: the corresponding probability for the χ^2-test statistics, i.e., which is equal to P(x> ChiSquare).

3.3 Anchoring

Once the functionality is initiated, anchoring information is first displayed on a top right window. For the example shown in Figure 3.2, Act8A and MWG912 are in anchor group 1. They were listed in Anchor1[2], the number in brachets is the number of marker in this anchor group.

Before Grouping is used, the users can manage the anchor information. If one wants to add the 5th marker ABG464 to the anchor group 2, right click by pointing the corresponding, and select the anchor group (Figure 3.2). ABG will be anchored to the second group, and the corresponding row will be highlighted (Figure 2.3). On the other side, one can also remove the anchor information by right clicking at the maker, and then select De-anchor, or move the marker to other anchor group (Figure 2.3).

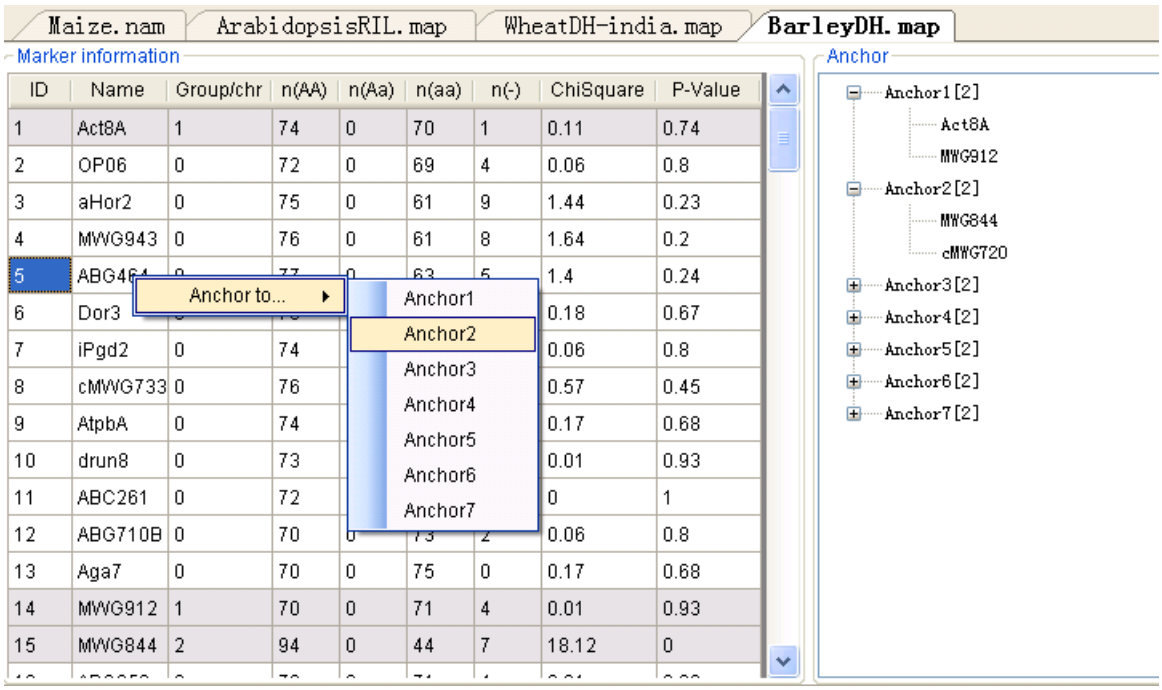


Figure 3.2 Adding markers to existing anchor groups

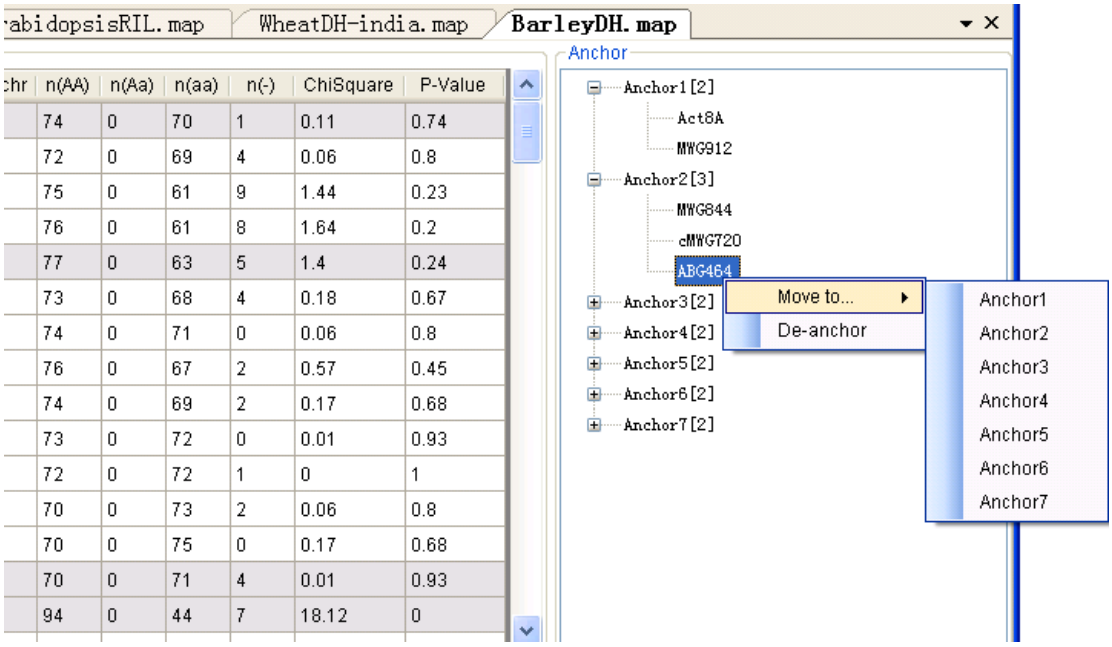


Figure 3.3 Moving anchor information or removing anchor information (De-anchor)

3.4 Grouping

After all anchor information is correctly managed, one can click the Grouping button so that all other unanchored markers are properly grouped (Figure 3.4). A LOD threshold, or a distance threshold or both criteria can be used in grouping the anchored marker.

- LOD threshold: the threshold of LOD score to declare the different linkage group. Any two markers with a LOD lower than the threshold will be grouped together.
- Distance threshold (cM): the threshold of distance between two markers to declare the different linkage group. Any two markers with a distance lower than the threshold will be grouped together.

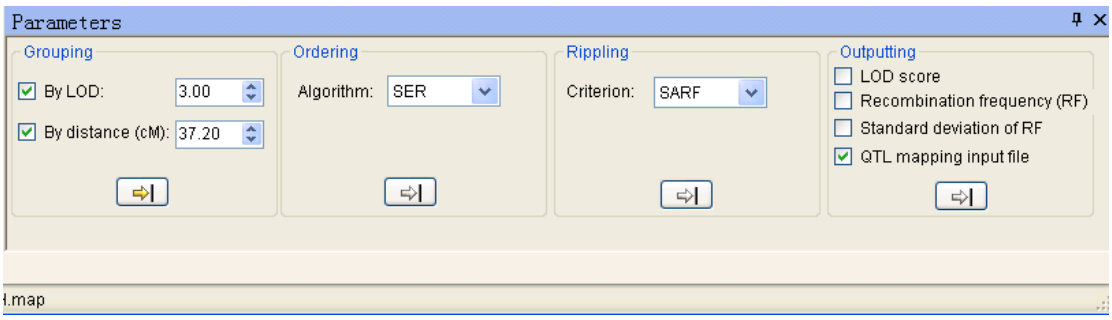
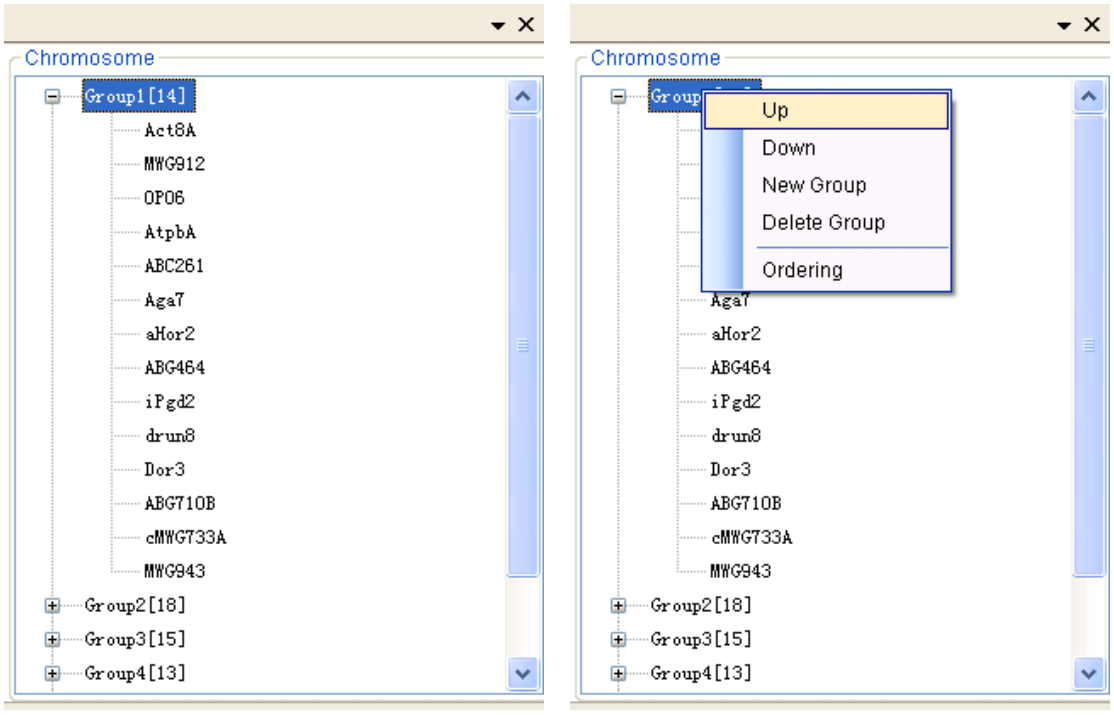


Figure 3.4 The parameter window in the MAP functionality

If neither criterion is selected, all unanchored markers will form one new chromosome. The number of chromosome groups does need to be equal to the number of anchor groups.

A. The Linkage Map Results Window after Grouping

B. Managing the grouped chromosomes



C. Managing markers in grouped chromosomes

D. Managing deleted markers

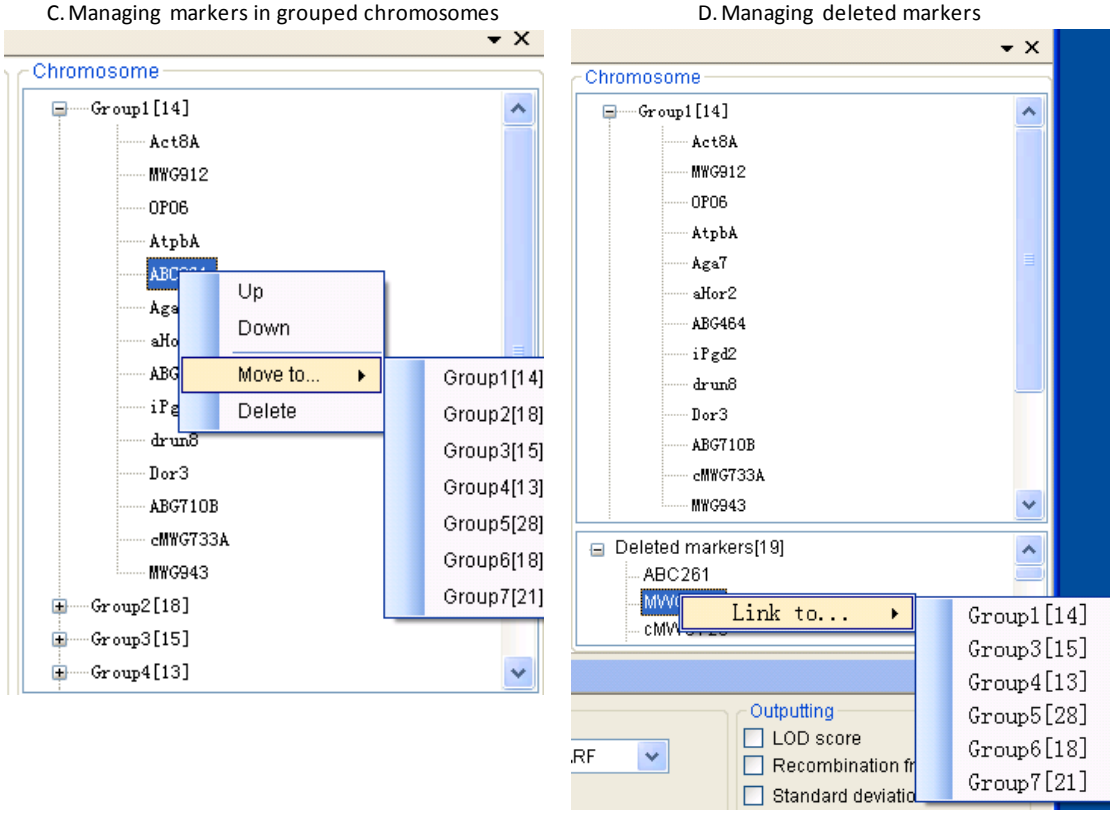


Figure 3.5 The Linkage Map Results Window after clicking the Grouping button

Additonal groups other than the achor groups may be generated if some unanchored markers cannot be grouped with any anchor group (Figure 3.5A). By clicking the right mouse button at a grouped chromsome, you can adjust the chromosome order, build a new chromosome group, delete the current chromosome, or conduct the ordering for markers within the chromosome (Figure 3.5B). By clicking the right mouse button at a marker, you can adjust the marker order, move the marker to other chromosome group, or delete the current marker (Figure 3.5C).

When any chromosomes or markers are delected, those markers will be shown in "Deletded markers" below the Chromsome Display Window (Figure 3.5D). By clicking the right mouse button at a deleted marker, you can link it to any exiting chromosome group (Figure 3.5D).

3.5 Ordering

After all markers are correctly grouped, one can click the Ordering button to make the genetic linkage maps (Figure 3.6A).

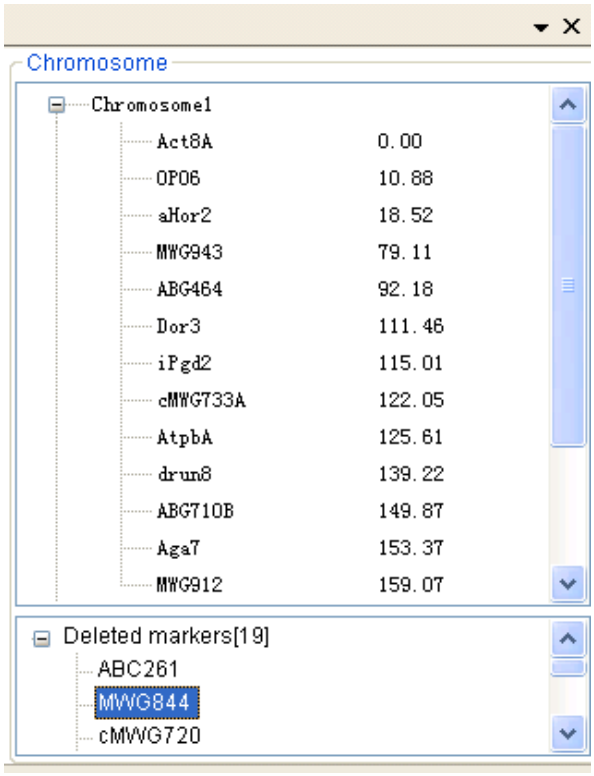
Three ordering algorithms are available.

- SER: seriation algorithm with details in Section 1.3
- RECORD: recombination counting and ordering algorithm with details in Section 1.3
- MF: multi-fragment algorithm with details in Section 1.3

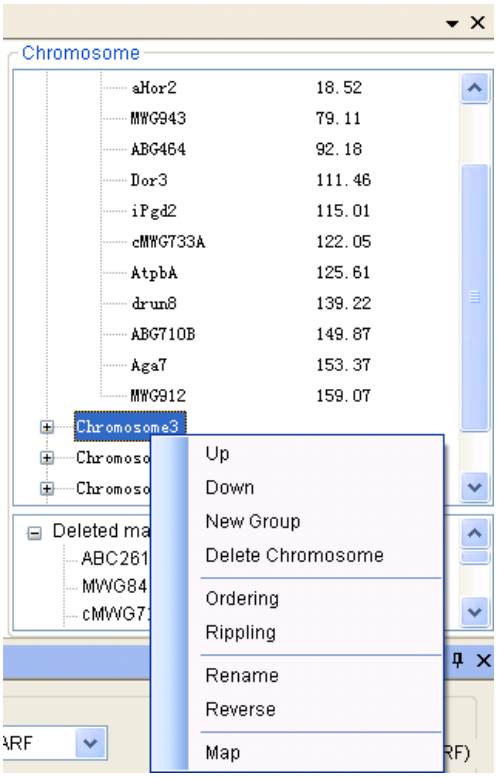
By clicking the right mouse button at an ordered chromsome, you can adjust the chromosome order by selecting Up or Down, build a new chromosome group, delete the current chromosome, conduct ordering or rippling for markers within the chromosome, rename or reverse the current chrosmome or draw the linkage map (Figure 3.6B). By clicking the right mouse button at a marker, you can adjust the marker order, move the marker to other chromosome group, or delete the current marker (Figure 3.6C). By clicking the right mouse button at a deleted marker, you can link it to any ordered chromosome (Figure 3.6D).

As shown in Figures 3.5B and 3.6B, each group can be ordered sepatatedly by right clicking, as well.

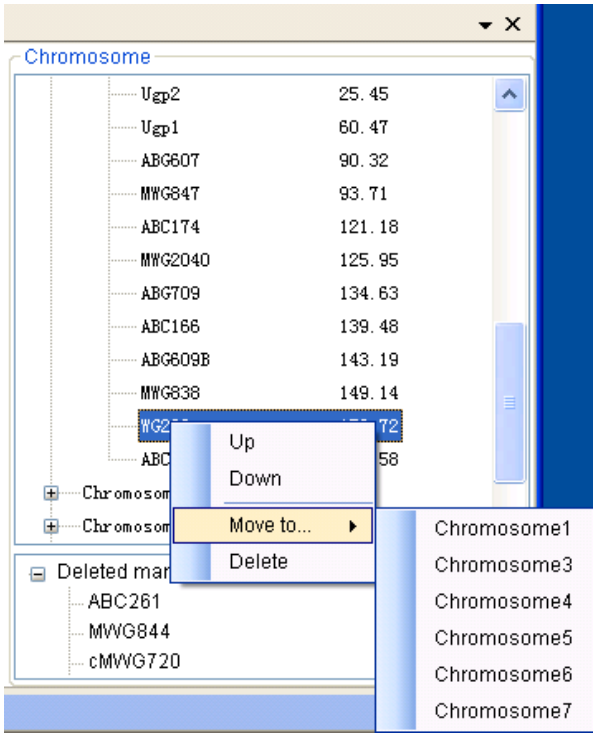
A. The Linkage Map Results Window after Ordering



B. Managing the ordered chromosomes



C. Managing markers in ordered chromosomes



D. Managing deleted markers

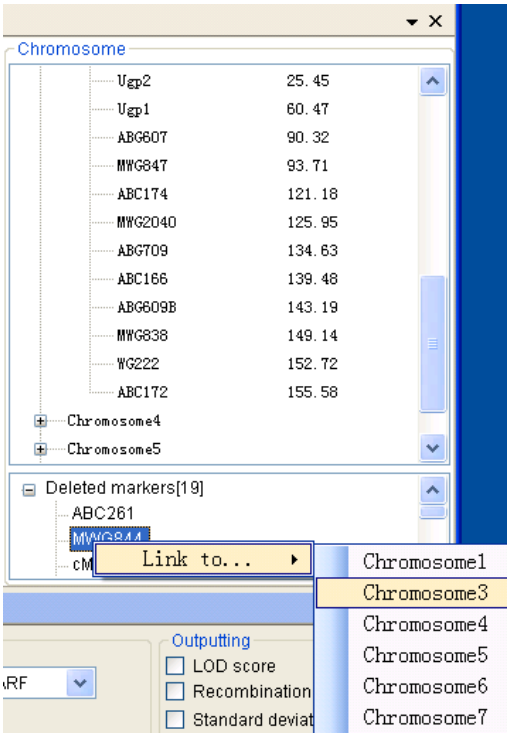


Figure 3.6 The Linkage Map Results Window after clicking the Ordering button

3.6 Rippling

Rippling is used as fine-tunning of the ordered chromosomes. Generally, the shorter the chromosome length, the better of the rippling results. Similar operations as shown in Figure 3.6 are available. As shown in Figure 3.6B, each group can be ordered sepatatedly by right clicking. Criteria used in rippling are

- SARF: sum of adjacent recombination fractions with details in Section 1.3
- SALOD: sum of adjacent LOD scores with details in Section 1.3
- SAD: sum of adjacent distances with details in Section 1.3
- COUNT: sum of the number of recombinants with details in Section 1.3
- LogL: sum of the logarithm likelihood under alternative hypothesis with details in Section 1.3

Similar to Ordering, by clicking the right mouse button at an ordered chromsome, you can adjust the chromosome order by selecting Up or Down, build a new chromosome group, delete the current chromosome, conduct ordering or rippling for markers within the chromosome, rename or reverse the current chrosmome or draw the linkage map (Figure 3.6B). By clicking the right mouse button at a marker, you can adjust the marker order, move the marker to other chromosome group, or delete the current marker (Figure 3.6C). By clicking the right mouse button at a deleted marker, you can link it to any ordered chromosome (Figure 3.6D).

As shown in Figures 3.5B and 3.6B, each group can be rippled sepatatedly by right clicking, as well.

3.7 Outputting

Outputting button is available after all chromosome grouped are properly ordered/rippled. Up to six files can be outputted by clicking the Outputting button in Figure 3.4.

3.7.1 TXT file: Linkage map information

The pure text file with the extension name TXT contains the linkage map information (Table 3.4) and some summary information for the map built (Table 3.5).

Table 3.4 shows the map for the first two chromosomes.

- Chromosome ID: ID number starting from 1 for each chromosome
- ChromosomeName : name of each chromosome
- MarkerName: name of each marker
- Interval (cM): length of the interval between one marker and its next

neighboring marker

- Position (cM): the position of the current marker in the linkage group

Table 3.4 Linkage map output (incomplete)

ChromosomeID	ChromosomeName	MarkerName	Interval(cM)	Position(cM)
1	Chromosome1	Act8A	10.88	0.00
1	Chromosome1	OP06	7.64	10.88
1	Chromosome1	aHor2	60.59	18.52
1	Chromosome1	MWG943	13.07	79.11
1	Chromosome1	ABG464	19.28	92.18
1	Chromosome1	Dor3	3.55	111.46
1	Chromosome1	iPgd2	7.04	115.01
1	Chromosome1	cMWG733A	3.56	122.05
1	Chromosome1	AtpbA	13.61	125.61
1	Chromosome1	drun8	10.65	139.22
1	Chromosome1	ABG710B	3.50	149.87
1	Chromosome1	Aga7	5.70	153.37
1	Chromosome1	MWG912	0.00	159.07
2	Chromosome3	ABC171	5.76	0.00
2	Chromosome3	CDO395	11.59	5.76
2	Chromosome3	ABG471	8.10	17.35
2	Chromosome3	Ugp2	35.02	25.45
2	Chromosome3	Ugp1	29.85	60.47
2	Chromosome3	ABG607	3.39	90.32
2	Chromosome3	MWG847	27.47	93.71
2	Chromosome3	ABC174	4.77	121.18
2	Chromosome3	MWG2040	8.68	125.95
2	Chromosome3	ABG709	4.85	134.63
2	Chromosome3	ABC166	3.71	139.48
2	Chromosome3	ABG609B	5.95	143.19
2	Chromosome3	MWG838	3.58	149.14
2	Chromosome3	WG222	2.86	152.72
2	Chromosome3	ABC172	0.00	155.58

…

Table 3.5 shows the summary information for the map.

- ChromosomeID: Chromosome ID represented by an integer number
- ChromosomeName: the name of each chromosome
- NumMarkers: the number of markers in each chromosome
- Length (cM): the length of each chromosome

The last two lines give the number of all markers, totoal chromosomal length, and number of deleted or unused markers.

Table 3.5 Summary of the linkage map

ChromosomeID	ChromosomeName	NumMarkers	Length(cM)
1	Chromosome1	13	159.07
2	Chromosome3	15	155.58
3	Chromosome4	13	189.70
4	Chromosome5	28	303.17
5	Chromosome6	18	160.85
6	Chromosome7	21	185.07
WholeGenome	WholeGenome	108	1153.44
DeletedMarkers	DeletedMarkers	19	0.00

3.7.2 LOD file: LOD scores matrix between markers

The file with extension name LOD contains the LOD between any pair of two markers. Part content in this file is given in Table 3.6.

- MarkerID: Marker ID represented by an integer number.
- MarkerName: The name of each marker.
- First row: Number after MarkerID and MarkerName is Marker ID as well.

Table 3.6 LOD scores between markers (incomplete)

MarkerID	MarkerName	1	2	3	4	5	6 …
1	Act8A	0.0000	21.4414	20.1871	0.7762	0.0766	0.2236
2	OP06	21.4414	0.0000	24.3561	0.5914	0.0256	0.1920
3	aHor2	20.1871	24.3561	0.0000	0.7457	0.2008	0.1978
4	MWG943	0.7762	0.5914	0.7457	0.0000	17.9595	4.3573
5	ABG464	0.0766	0.0256	0.2008	17.9595	0.0000	12.7581
6	Dor3	0.2236	0.1920	0.1978	4.3573	12.7581	0.0000

…

3.7.3 REC file: recombination frequency matrix between markers

The file with extension name REC contains the recombination frequency between any pair of two markers. Part content in this file is given in Table 3.7.

- MarkerID: Marker ID represented by an integer number.
- MarkerName: The name of each marker.
- First row: Number after MarkerID and MarkerName is Marker ID as well.

Table 3.7 Recombination frequency between markers (incomplete)

MarkerID	MarkerName	1	2	3	4	5	6 …
1	Act8A	0.0000	0.1071	0.1111	0.4191	0.4748	0.4571
2	OP06	0.1071	0.0000	0.0758	0.4286	0.4853	0.4599
3	aHor2	0.1111	0.0758	0.0000	0.4186	0.4580	0.4586
4	MWG943	0.4191	0.4286	0.4186	0.0000	0.1278	0.3083
5	ABG464	0.4748	0.4853	0.4580	0.1278	0.0000	0.1838
6	Dor3	0.4571	0.4599	0.4586	0.3083	0.1838	0.0000

…

3.7.4 STD file: standard deviation matrix between markers

The file with extension name STD contains standard error of the estimated recombination frequency between any pair of two markers. Part content in this file is given in Table 3.8.

- MarkerID: Marker ID represented by an integer number.
- MarkerName: The name of each marker.
- First row: Number after MarkerID and MarkerName is Marker ID as well.

Table 3.8 Standard error of the estimated recombination frequency between markers (incomplete)

MarkerID	MarkerName	1	2	3	4	5	6 …
1	Act8A	0.0000	0.0007	0.0007	0.0018	0.0018	0.0018
2	OP06	0.0007	0.0000	0.0005	0.0018	0.0018	0.0018
3	aHor2	0.0007	0.0005	0.0000	0.0019	0.0019	0.0019
4	MWG943	0.0018	0.0018	0.0019	0.0000	0.0008	0.0016
5	ABG464	0.0018	0.0018	0.0019	0.0008	0.0000	0.0011
6	Dor3	0.0018	0.0018	0.0019	0.0016	0.0011	0.0000

…

3.7.5 MTP file: marker summary and marker types

The file with extension name MTP contains some marker summary information and marker types. Markers are in the order as in the linkage map. Part content in this file is given in Table 3.9.

- MarkerID: Marker ID represented by an integer number.
- MarkerName: name of each marker
- Chromosome ID: ID number starting from 1 for each chromosome
- ChromosomeName : name of each chromosome
- Position (cM): the position of the current marker in the linkage group
- n(AA): the number of genotypes same as the first parent (P1), which denoted as 2 in the input file.
- n(Aa): the number of genotypes same as F1, which denoted as 1 in the input file.
- n(aa): the number of genotypes same as the second parent (P2), which denoted as 0 in the input file.
- n(-): the number of missing genotypes, which denoted as -1 in the input file.
- ChiSquare: χ^2-test statistics testing for segregation distortion of markers.
- P-Value: the corresponding probability for the χ^2-test statistics, i.e., which is equal to P(x> ChiSquare).

Table 3.9 Marker summary and marker types (incomplete)

MarkerID	MarkerName	Chromosome	Position	n(AA)	n(Aa)	n(aa)	n(-)	Chi^2-Test	P(x>Chi^2)
1	Act8A	1	0.00	74	0	70	1	0.11	0.74
2	OP06	1	10.88	72	0	69	4	0.06	0.8
3	aHor2	1	18.52	75	0	61	9	1.44	0.23
4	MWG943	1	79.11	76	0	61	8	1.64	0.2
5	ABG464	1	92.18	77	0	63	5	1.4	0.24
6	Dor3	1	111.46	73	0	68	4	0.18	0.67
7	iPgd2	1	115.01	74	0	71	0	0.06	0.8
8	cMWG733A	1	122.05	76	0	67	2	0.57	0.45
9	AtpbA	1	125.61	74	0	69	2	0.17	0.68
10	drun8	1	139.22	73	0	72	0	0.01	0.93
11	ABG710B	1	149.87	70	0	73	2	0.06	0.8
12	Aga7	1	153.37	70	0	75	0	0.17	0.68
13	MWG912	1	159.07	70	0	71	4	0.01	0.93
14	ABC171	2	0.00	82	0	62	1	2.78	0.1
15	CDO395	2	5.76	67	0	56	22	0.98	0.32
16	ABG471	2	17.35	72	0	73	0	0.01	0.93
17	Ugp2	2	25.45	65	0	72	8	0.36	0.55
18	Ugp1	2	60.47	52	0	38	55	2.18	0.14
19	ABG607	2	90.32	71	0	61	13	0.76	0.38
20	MWG847	2	93.71	69	0	61	15	0.49	0.48
21	ABC174	2	121.18	68	0	75	2	0.34	0.56
22	MWG2040	2	125.95	59	0	69	17	0.78	0.38
23	ABG709	2	134.63	71	0	74	0	0.06	0.8
24	ABC166	2	139.48	72	0	73	0	0.01	0.93
25	ABG609B	2	143.19	66	0	69	10	0.07	0.8
26	MWG838	2	149.14	72	0	73	0	0.01	0.93
27	WG222	2	152.72	70	0	70	5	0.00	1.0
28	ABC172	2	155.58	71	0	74	0	0.06	0.8

```
!************************ Marker Types ***********************************************
!Marker type: 2 for P1; 1 for F1; 0 for P2; -1 for missing markers
 Act8A   0  2 -1  2  0  2  2  0  0  2  2  2  0  2  2  0  0  0  2  2  0  0  2…
 OP06    0  2  2  2  0  2  2  0  0  2  2  2  0  2  2  0  0  2  2  2  0  0  2…
 aHor2   0  2  2  2  0  2  2  0  0  2  2  2  0  2  2  0  0  2  2  2  0  0  2…
 MWG943  2  2  2  2  2  2  0  0  0  0  2  0  2  0  2  2  0  2  2  0  0  2  2…
```

…

3.7.6 BIP file: The input file for QTL mapping in QTL IciMapping

The file with extension name BIP has the exact same format as the file used in QTL mapping for biparental populations. By adding the phenotypic data, this output file is ready for the BIP functionality, which will be desricbed in details in next chapter.

3.8 Draw linkage maps

Click the MAP tool bar 

or Linkage map in the Figures menu to draw the linkage map. A new window with the title "[Graph] Linkage Map window" will be open for drawing the linkage maps. The linkage map drawing can be conducted one by one chromosome (Figure 3.7A), or for all chrosmomes (Figure 3.7B), or for selected chromosomes (Figure 3.8).

Six tool bars in the [Graph] Linkage Map window are:

- 

: save the graph in various formats
- 

: print out the graph
- 

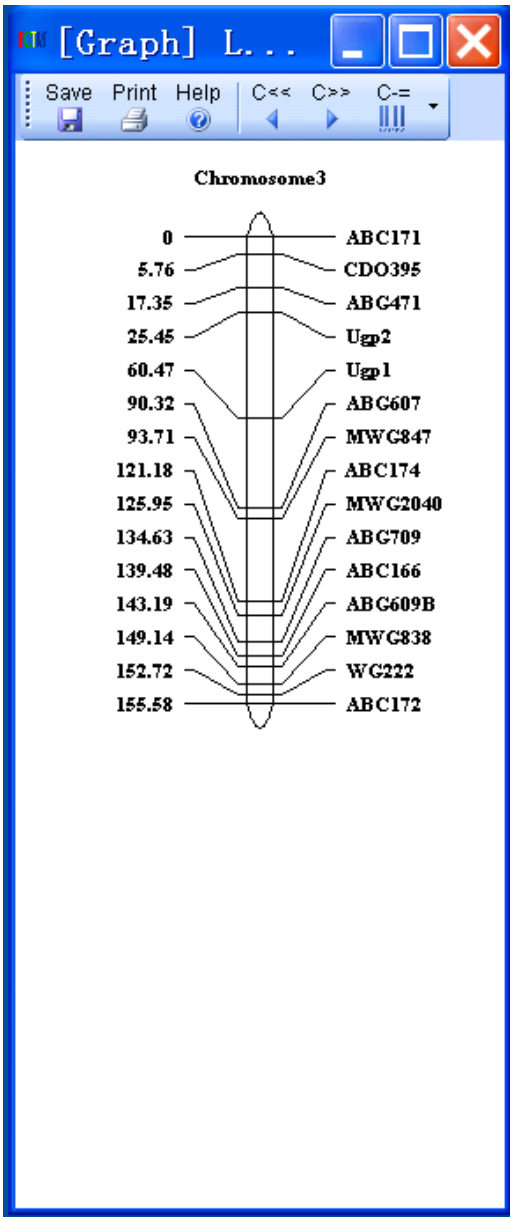
: open QTL IciMapping Users' Manual
- 

: draw linkage map for the next chromosome
- 

: draw linkage map for the previous chromosome
- 

: draw linkage maps for all the chromosomes or selected chromosomes

A. Map of one chromosome



B. Map of all chromosomes

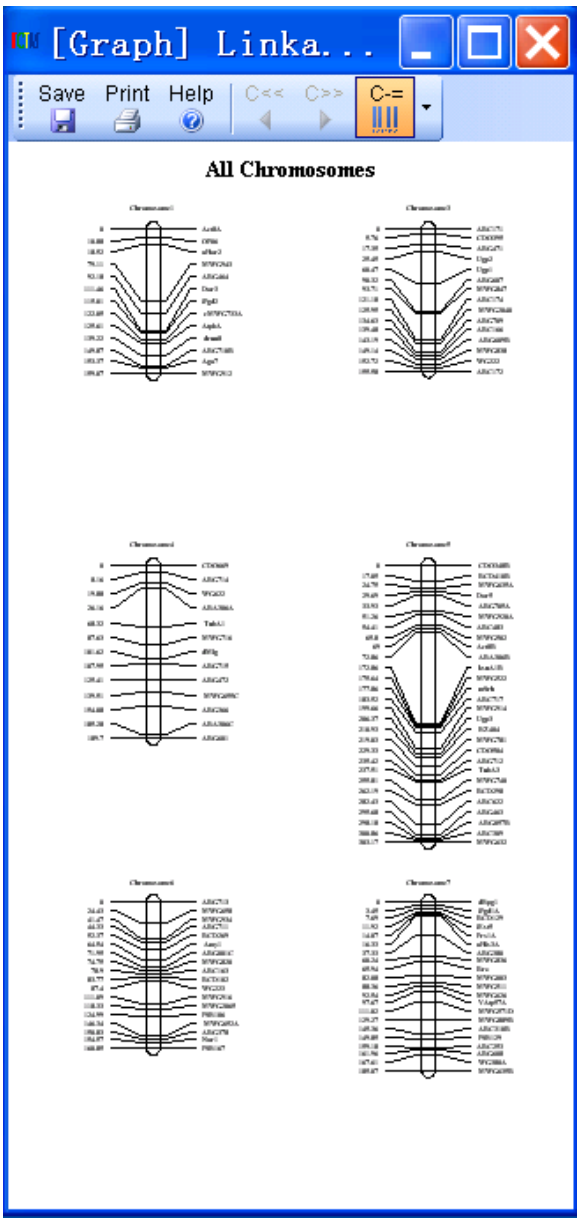


Figure 3.7 Linkage map for each chromosome or all chrosmosomes

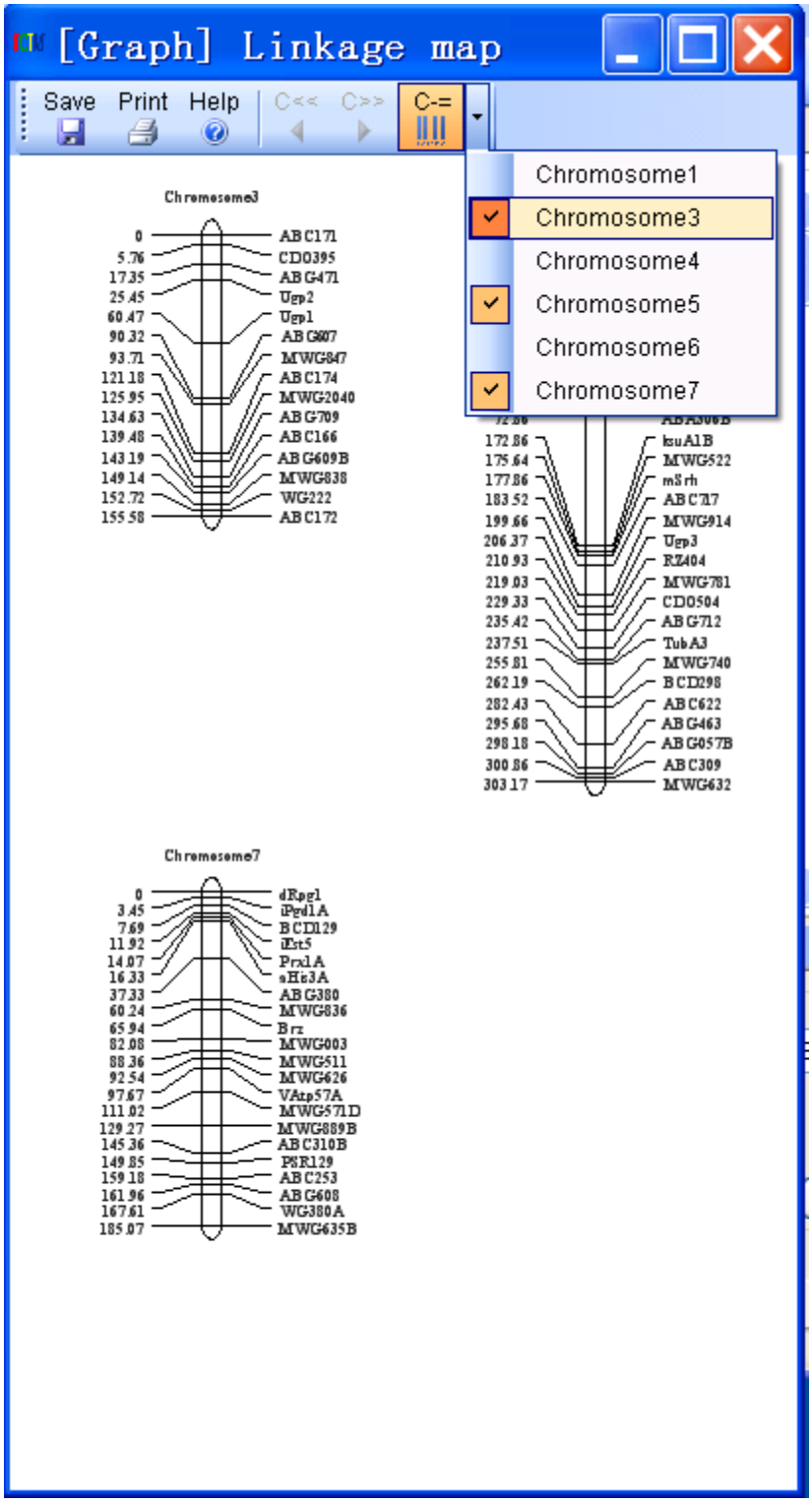


Figure 3.8 Linkage map for selected chrosmosomes

Chapter 4. QTL Mapping in Biparental Populations (BIP)

By using QTL IciMapping, one can conduct QTL mapping for actual marker and phenotypic data in biparental populations (i.e. Backcross, DH, RIL and F_2 and so on; Figure 1.1). In this chapter we will introduce QTL mapping for standard biparental populations. There are five mapping methods in the software: single marker analysis (SMA), simple interval Mapping (SIM), ICIM for additive mapping (ICIM-ADD), ICIM for epistatic mapping (ICIM-EPI) and selective genotyping mapping (SGM).

One mapping population is defined in an input file with the extension name 'bip'. Tables 4.1-4.5 give a working example for an F_2 population with 180 individuals. Five parts can be seen in one BIP input file, i.e. the general information for the mapping population (Table 4.1), linkage groups (Table 4.2), definition of each chromosome (Table 4.3), marker types (Table 4.4), and phenotypes (Table 4.5). Lines starting with '!' are remarks and will be ignored in the QTL IciMapping software.

4.1 Input file for QTL mapping in biparental populations (*.bip)

4.1.1 General information of the mapping population

Eight parameters were used for the general information defining a linkage mapping population (Table 4.1).

- Indicator: This indicator lets IciMapping know if a mapping study or power simulation will be conducted. Indicator 1 has to be assigned for any QTL mapping study, and 2 for power analysis (Table 4.1). The input file for QTL mapping is not completely the same as that for power analysis.
  - 1 for a mapping study
  - 2 for power simulation

- Population Type: describe the type of the population. At present, QTL IciMapping can conduct linkage map construction for twenty populations derived from two parental lines (Figure 1.1). Assuming F1 = P1 x P2, the 20 biparental populations are:
  - 1. P1BC1F1: the backcross population where the first parent (P1) is used as the recurrent.
  - 2. P2BC1F1: the backcross population where the second parent (P2) is used as the recurrent.
  - 3. F1DH: doubled haploids derived from F1.
  - 4. RIL: recombination inbred lines derived from repeated selfing since F1 generation.
  - 5. P1BC1RIL: recombination inbred lines derived from the backcross population where the first parent is used as the recurrent.

- 6. P2BC1RIL: recombination inbred lines derived from the backcross population where the second parent is used as the recurrent.
- 7. F2: the selfing generation of F1.
- 8. F3: the selfing generation of F2.
- 9. P1BC2F1: the second backcrossing where P1 is used as the recurrent parent.
- 10. P2BC2F1: the second backcrossing where P2 is used as the recurrent parent.
- 11. P1BC2RIL: recombination inred lines through the repeated selfing of P1BC2F1.
- 12. P2BC2RIL: recombination inred lines through the repeated selfing of P2BC2F1.
- 13. P1BC1F2: the selfing generation of P1BC1F1.
- 14. P2BC1F2: the selfing generation of P2BC1F1.
- 15. P1BC2F2: the selfing generation of P1BC2F1.
- 16. P2BC2F2: the selfing generation of P2BC2F1.
- 17. P1BC1DH: P1BC1F1-derived doubled haploids.
- 18. P2BC1DH: P2BC1F1-derived doubled haploids.
- 19. P1BC2DH: P1BC2F1-derived doubled haploids.
- 20. P2BC2DH: P2BC2F1-derived doubled haploids.

- Mapping Function: specify the mapping function which will be used to transfer recombination frequency to mapping distance, or from mapping distance to recombination frequency.
  - 1 for Kosombi mapping function.
  - 2 for Haldane mapping function.
  - 3 for Morgan mapping function.

- Marker Space Type: specify whether the markers on a chromosome (or linkage group) are defined by positions or marker intervals.
  - 1 for intervals, i.e. the number behind a marker is the distance of the marker to its next marker. 0 is normally given for the last marker on a chromosome or a linkage group.
  - 2 for positions, i.e. the number behind each marker is the position of the marker on the chromosome or the linkage group.

- Marker Space Unit: specify the unit used in marker linkage group.
  - 1 for centi-Morgan (cM).
  - 2 for Morgan (M). 1 M = 100 cM.

- Number of Chromosomes: specify the number of chromosomes (or linkage groups) in the mapping population.

- Population Size: number of individuals in the mapping population.

- Number of Traits: number of traits phenotyped in the mapping population.

Table 4.1 General information in a QTL mapping input file

```
!***** Note: lines staring with "!" are remarks and will be ignored in the program***
!***** General Information ***********************************************************
!Assuming F1 = P1 x P2, populations available in QTL IciMapping are:
!  1, P1BC1F1 = P1 x F1, the first backcrossing where P1 is used as the recurrent parent;
!  2, P2BC1F1 = P2 x F1, the first backcrossing where P2 is used as the recurrent parent;
!  3, F1DH, F1-derived doubled haploids;
!  4, RIL or F1RIL, recombination inbred lines through the repeated selfing of F1;
!  5, P1BC1RIL, recombination inbred lines through the repeated selfing of P1BC1F1;
!  6, P2BC1RIL, recombination inbred lines through the repeated selfing of P2BC1F1;
!  7, F2, the selfing generation of F1;
!  8, F3, the selfing generation of F2;
!  9, P1BC2F1, the second backcrossing where P1 is used as the recurrent parent;
! 10, P2BC2F1, the second backcrossing where P2 is used as the recurrent parent;
! 11, P1BC2RIL, recombination inbred lines through the repeated selfing of P1BC2F1;
! 12, P2BC2RIL, recombination inbred lines through the repeated selfing of P2BC2F1;
! 13, P1BC1F2, the selfing generation of P1BC1F1;
! 14, P2BC1F2, the selfing generation of P2BC1F1;
! 15, P1BC2F2, the selfing generation of P1BC2F1;
! 16, P2BC2F2, the selfing generation of P2BC2F1;
! 17, P1BC1DH, P1BC1F1-derived doubled haploids;
! 18, P2BC1DH, P2BC1F1-derived doubled haploids;
! 19, P1BC2DH, P1BC2F1-derived doubled haploids;
! 20, P2BC2DH, P2BC2F1-derived doubled haploids;
   1     !Indicator: 1 for mapping; 2 for simulation
   7     !Mapping Population Type (see remarks above)
   1     !Mapping Function (1 for Kosambi; 2 for Haldane; 3 for Morgan)
   1     !Marker Space Type (1 for intervals; 2 for positions)
   1     !Marker Space Unit(1 for centiMorgan; 2 for Morgan)
  12     !Number of Chromosomes (or Linkage Group)
 180     !Population size of the mapping population
   1     !Number of traits
```

4.1.2 Linkage group or chromosome information

The name of each chromosome and the number of markers on the chromosome were specified first (Table 4.2), followed by the definition of each chromosome (Table 4.3). Each chromosome was defined by all markers on it and all marker positions.

Table 4.2 Linkage group information in a QTL mapping input file

```
!***********************Information for Chromosomes and Markers***********************
!Chromosome     NumMarkers in each chromosome
Ch1              11
Ch2              14
Ch3              7
Ch4              13
Ch5              7
Ch6              9
Ch7              9
Ch8              10
Ch9              9
Ch10             9
Ch11             11
Ch12             8
```

Table 4.3 Definition of each chromosome

```
!Linkage map (Marker name followed by position or the interval length)
RM1-004       1    35.8
RM1201        1    4.3
RM490         1    10.3
RM576         1    10.5
RM600         1    10.9
RM562         1    4
RM449         1    3.6
RM466         1    14.6
RM493         1    26.4
RM488         1    38.5
RM1003        1    0
```

RM233A	2	5.3
RM5529	2	34.8
RM1358	2	3.9
RM3549	2	2.9
RM5791	2	0.9
RM5390	2	1.1
RM424	2	42.9
RM6318	2	13.4
RM525	2	22.8
RM8255	2	5.5
RM240	2	5.2
RM250	2	2.3
RM2_117	2	12.5
RM207	2	0
RM81B	3	37.5
RM3467	3	12
RM3766	3	22.6
RM251	3	35.7
RM411	3	8.3
RM16	3	40.3
RM7389	3	0
RM401	4	7.3
RM335	4	22.1
RM4_62	4	0.3
RM471	4	10.8
RM1359	4	5.3
RM1155	4	5.8
RM5635	4	3.5
RM119	4	40
RM6748	4	6.2
RM5473	4	2.5
RM348	4	0.9
RM349	4	31.5
RM559	4	0
RM5_6	5	7.9
RM5_9	5	7.2
RM5_11	5	9.7
RM5_14	5	18.1
RM5_13	5	16.2
RM5_21	5	34.5
RM5_31	5	0
RM6_2	6	1.9
RM6_7	6	18
RM6_13	6	5.5
RM6_17	6	0.3
RM6_19	6	31.7
RM6_30	6	1.2
RM6_33	6	1.5
RM6_34	6	32.7
RM6_42	6	0
RM295	7	32.8
RM501	7	8.5
RM542	7	4.2
RM500	7	22.4
RM346	7	0.5
RM336	7	3.8
RM455	7	5.5
RM6344	7	34.5
RM248	7	0
RM152	8	17.1
RM3702	8	37.6
RM72	8	5.7
RM547	8	34.3
RM210	8	7.3
RM5485	8	22.8
RM6948	8	11.6
RM6765	8	6.1
RM264	8	6.7
RM281	8	0
RM1328	9	13.2
RM105	9	5.7
RM2038	9	34.4
RM434	9	11.8
RM3533	9	8.3
RM242	9	6.1
RM201	9	27.9

```
RM328         9    3.7
OSR28         9    0
RM5095        10   13.4
RM222         10   9.5
RM6646        10   21
RM5620        10   28.1
RM258         10   16.8
RM294         10   17.8
RM590         10   4
RM333         10   7.3
RM496         10   0
RM286         11   25
RM7557        11   7
RM332         11   12.7
RM5704        11   4.5
RM6894        11   2.8
RM167         11   13.4
RM202         11   2.7
RM287         11   2.7
RM229         11   3.9
RM21          11   33.4
RM3117        11   0
RM4           12   11.5
RM19          12   17.4
RM247         12   11.8
RM6296        12   21.3
RM1246        12   8.3
RM519         12   36.1
RM7120        12   36.4
RM12          12   0
```

4.1.3 Marker type information

Marker types are arranged by the ordered markers defined in Table 4.3. That is, the marker types for all individuals on the first marker were given first, then followed by the second marker, and so on (Table 4.4). The marker name in this part has to be the same as that specified in Table 4.3. In IciMapping, 2 was used to represent the marker type of the first parent (P1), 0 for the second parent (P2), 1 for the F1 marker type, and -1 for any missing markers. Any missing markers will be assigned values based on the types of their neighboring markers. The number of marker types behind each marker name has to be exact the population size.

Table 4.4 Marker type information in a QTL mapping input file (incomplete)

```
!**********************************Marker Type**************************************
!Marker type: 2 for P1; 1 for F1; 0 for P2; BC1=F1xP1; BC2=F1xP2; -1 for missing markers if any.
RM1-004  1   0   0   2   1   2   2   2   1   1   0   1   1   0   -1  1   2   0
     2   0   1   1   -1  1   -1  -1  1   1   2   1   2   1   2   2   2   1   -1
     1   1   1   -1  2   2   1   1   1   2   1   1   -1  1   0   2   0   2   1
     2   2   0   2   -1  1   -1  2   1   0   -1  0   -1  1   1   1   1   -1  -1
     1   1   0   2   1   1   2   1   1   -1  2   2   1   0   1   1   1   1   2
     1   1   1   0   2   1   1   2   1   1   0   1   0   1   1   1   0   2   1
     2   1   2   0   1   1   1   1   1   2   1   2   2   0   0   1   1   1   2
     1   0   2   0   1   0   2   1   1   1   2   1   1   1   2   0   1   1   0
     1   1   0   2   1   1   1   1   2   1   0   1   1   2   1   2   2   0   0
     1   1   2   1   1   0   1   1   1   2
RM1201   1   -1  -1  1   1   1   1   0   1   1   0   1   1   0   0   1   1   1
     1   0   1   1   1   2   1   1   1   1   1   2   2   1   1   2   1   2   -1
     1   1   0   2   1   0   2   2   1   1   1   1   -1  2   0   2   2   2   1
     2   1   1   1   1   1   1   1   1   2   1   1   1   0   2   1   1   -1  1
     1   0   1   -1  1   1   2   0   1   -1  1   -1  -1  1   2   1   0   1   2
     1   1   1   1   2   1   1   1   1   2   0   1   0   0   0   1   1   2   1
     1   1   1   0   1   2   1   1   2   1   0   2   2   0   1   0   1   0   2
     1   1   2   1   1   1   2   1   1   1   2   1   1   2   1   1   0   1   0
     1   1   0   2   1   1   1   2   2   1   1   1   1   1   1   2   2   0   1
     1   1   2   2   1   0   1   2   1   2
RM490    1   1   -1  1   1   -1  1   0   1   1   0   1   1   0   0   -1  1   1
```

```
    1   -1   -1   1   1   2   -1   -1   1   -1   -1   -1   2   -1   2   1   1   2   -1
    1   1   0   1   2   0   2   2   1   -1   1   1   -1   -1   0   2   -1   2   1
    -1   1   -1   1   0   1   -1   1   1   2   1   1   1   0   -1   -1   1   2   1
    -1   -1   1   -1   -1   1   2   2   1   -1   1   -1   -1   1   2   -1   0   1   2
    1   1   1   1   2   1   1   1   2   2   0   1   0   0   0   1   1   2   1
    1   1   1   0   1   2   1   1   2   1   0   2   2   0   1   0   1   0   2
    1   1   2   2   1   1   2   1   1   1   2   1   1   2   1   1   0   0   0
    1   1   0   2   1   2   1   2   2   1   1   1   1   1   1   2   2   0   1
    1   1   2   2   1   0   1   2   1   2
……
```

4.1.4 Phenotype information

Phenotypes were first given for the first trait, then for the second trait and so on. -100.00 is reserved for any missing phenotype (Table 4.5). There is no fixed format for the number of values which can be seen in each line in Table 4.5. The user can arrange any number of phenotypic values in one line at their own preference.

Table 4.5 Phenotype information in a QTL mapping input file.

```
!***************************Phenotypic Data*************************************
!-100.0 is resevred for missing phneotype
Resistance   7.90     2.30     6.73     4.33     6.33     4.33     4.41     9.00
    9.00     6.28     3.55     0.83     9.00     5.10     6.88     8.68     2.89
    1.50     4.59     4.00     3.21     1.92     3.10     2.86     2.80     7.64
    5.83     3.69     1.92     5.13     1.33     5.63     0.00     3.67     9.00
    6.15     7.64     4.07     5.06     6.88     1.88     3.33     1.43     1.92
    1.40     1.67     3.44     8.80     7.50     0.00     3.33     3.33     2.89
    9.00     5.77     6.15     6.55     3.33     9.00     5.31     9.00     3.75
    5.00     4.00     9.00     4.60     6.36     6.60     4.38     3.08     7.33
    8.00     7.50     4.00     4.00     0.00     4.64     4.08     6.43     0.83
    3.58     2.14     0.33     4.67     3.69     5.51     0.00     5.12     6.33
    1.51     6.67     1.94     1.17     2.98     7.17     5.90     5.70     7.00
    3.68     4.17     4.29     2.16     6.79     1.56     2.64     2.08     4.68
    0.00     3.90     0.00     9.00     4.29     2.67     0.63     5.00     8.33
    4.13     3.64     1.43     7.69     1.74     4.29     4.15     4.43     2.99
    4.33     7.83     6.33     1.25     1.07     9.00     4.33     4.67     0.59
    9.00     0.00     1.63     1.92     5.35     6.51     2.50     0.00     5.63
    4.38     1.29     8.08     9.00     9.00     9.00     9.00     1.33     7.89
    7.00     7.50     4.09     9.00     3.33     9.00     9.00     9.00     2.72
    0.60     1.00     5.92     6.79     2.35     0.65     7.00     9.00     7.80
    5.33     8.27     4.97     7.50     1.19     4.33     0.00     9.00     6.21
    4.38
```

4.2 Input file for QTL mapping in the EXCEL format

One mapping population for QTL mapping in biparental populations can also be defined in an Excel file with the extension name ‘xls’ or ‘xlsx’. The file should be composed of five sheets: ‘GeneralInfo’ (similar to Table 4.1), ‘Chromosome’ (similar to Table 4.2), ‘LinkageMap’ (similar to Table 4.3), ‘Genotype’ (similar to Table 4.4), and ‘Phenotype’ (similar to Table 4.5).

4.3 Setting mapping parameters

The BIP functionality can be initiated by (1) opening input files, or (2) double clicking BIP files listed in the project window, or (3) clicking one BIP file in the display window (Figure 2.1). When functionality BIP is activated, the Display

window shows the contents of the current input file, and the Parameter window is for interaction with users (Figure 4.1).

Five mapping methods can be conducted in IciMapping, i.e. single marker analysis (SMA), the traditional inyterval mapping (SIM), ICIM for QTL with additive(and dominance) effects (or one dimensional scanning) (ICIM-ADD), ICIM for digenic QTL networks (or two dimensional scanning) (ICIM-EPI), and selective genotyping mapping (SGM). The threshold LOD score declaring significant additive QTL or QTL networks can be specified or determined from a number of permutation tests.

Once parameters are set for a method, to click  to select the defined method. to click  to unselect a method in the Selected Methods box (Figure 4.1).

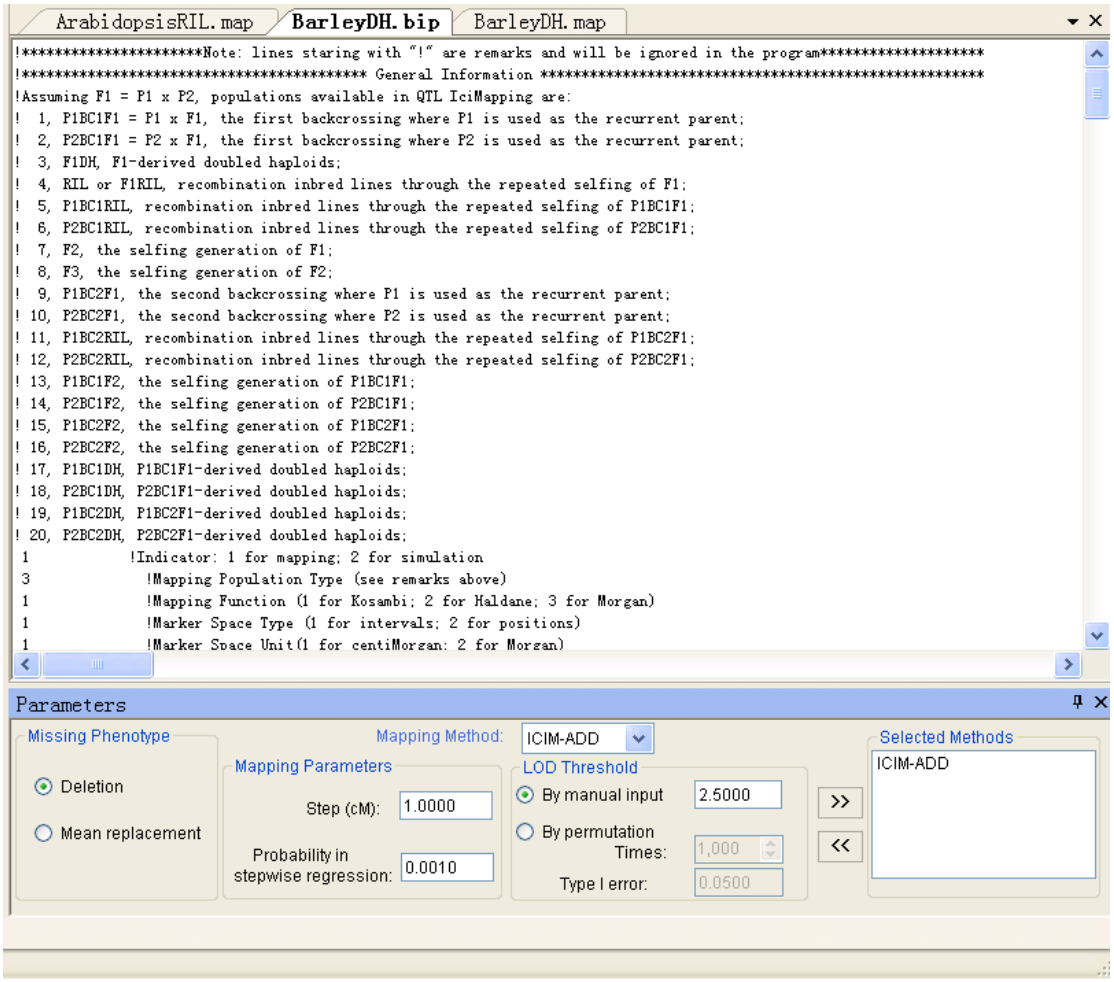


Figure 4.1 Display and Parameter windows in BIP functionality

4.3.1 Handling missing phenotype

Two options are available for handling missing phenotypic values (Figure 4.1).

- Deletion, i.e., the missing phenotypic data will not be included in QTL mapping.
- Mean replacement, i.e., the missing phenotypic data will be replaced by phenotypic mean of the trait.

4.3.2 Parmeters for SMA (Single Marker Analysis, Figure 4.2)

- LOD Threshold: the threshold LOD score to declare significant QTL.
  - By manual Input: determine a threshold LOD by user
  - By permutation: determine a threshold LOD by permutation
    - Times: number of permutation tests.
    - Type I error: type I error to determine the LOD threshold from permutation tests.

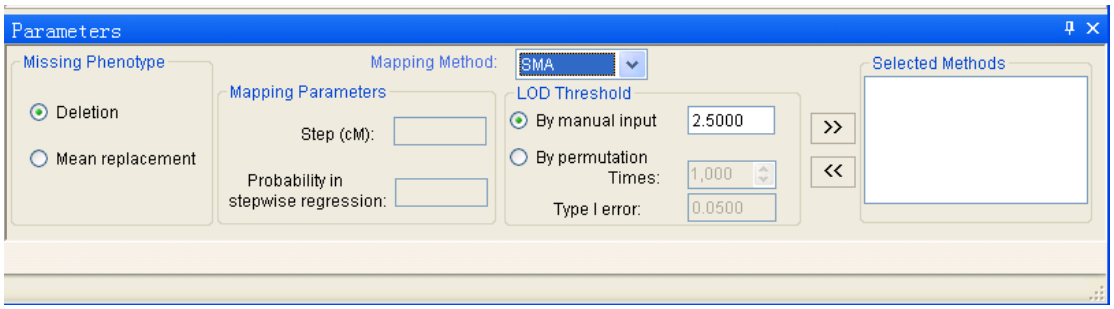


Figure 4.2 Parameters for SMA

4.3.3 Parameters for SIM (Simple Interval Mapping, Figure 4.3)

- Step (cM): the step in scanning represented by cM.
- LOD Threshold: the threshold LOD score to declare significant QTL.
  - By manual Input: determine a threshold LOD by user
  - By permutation: determine a threshold LOD by permutation
    - Times: number of permutation tests.
    - Type I error: type I error to determine the LOD threshold from permutation tests.

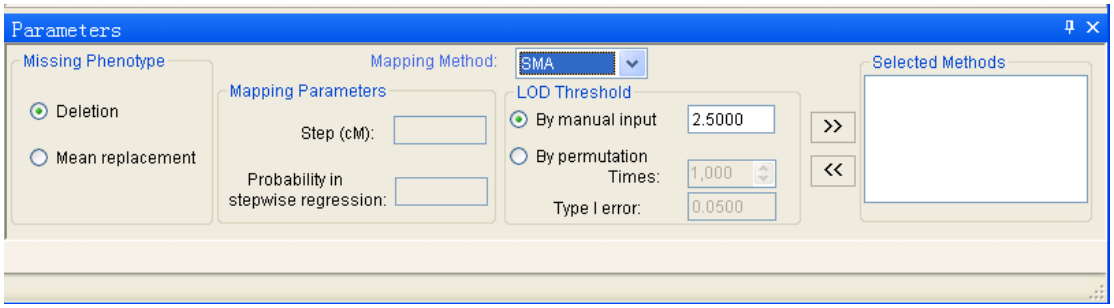


Figure 4.3 Parameters for SIM

4.3.4 Parameters for ICIM of QTL with additive (and dominance) effects or one dimensional ICIM (Abbreviated as ICIM-ADD, Figure 4.4)

- Step (cM): the step in scanning represented by cM.
- Probability in stepwise regression: the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables (PIN). The largest P-value for removing variables is assumed to be two times of PIN.
- LOD Threshold: the threshold LOD score to declare significant QTL.
    - By manual Input: determine a threshold LOD by user
    - By permutation: determine a threshold LOD by permutation
        - Times: number of permutation tests.
        - Type I error: type I error to determine the LOD threshold from permutation tests.

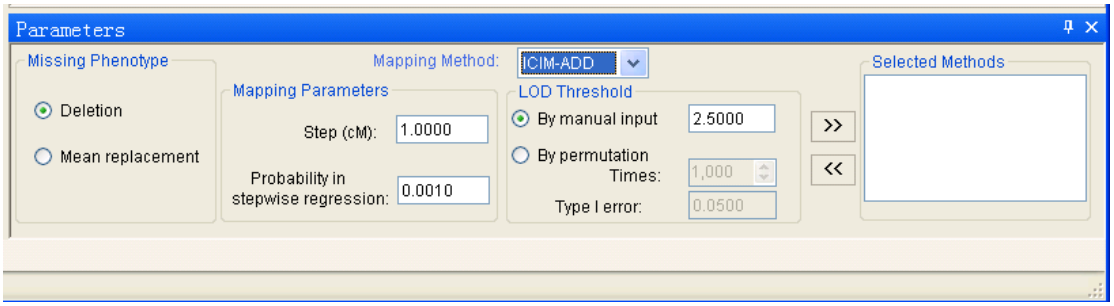


Figure 4.4 Parameters for ICIM-ADD

4.3.5 Parameters for ICIM of digenic QTL networks or two dimensional ICIM (Abbreviated as ICIM-EPI, Figure 4.5)

- Step (cM): the step in scanning represented by cM.
- Probability in stepwise regression: the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables (PIN). The largest P-value for removing variables is assumed to be two times of PIN.

- LOD Threshold: the threshold LOD score to declare significant QTL.
  - By manual Input: determine a threshold LOD by user
  - By permutation: determine a threshold LOD by permutation
    - Times: number of permutation tests.
    - Type I error: type I error to determine the LOD threshold from permutation tests.

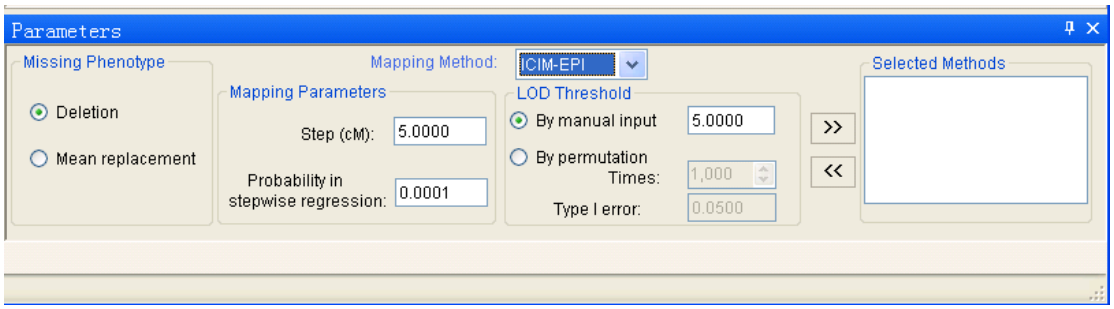


Figure 4.5 Parameters for ICIM-EPI

4.3.6 Parameters for SGM (Selective Genotyping Mapping, Figure 4.6)

- Tail: conducting SGM using the top tail, the bottom tail, or both tails.
- Bottom: proportion of the bottom tail.
- Top: proportion of the top tail.
- LOD Threshold: the threshold LOD score to declare significant QTL.
  - By manual Input: determine a threshold LOD by user
  - By permutation: determine a threshold LOD by permutation
    - Times: number of permutation tests.
    - Type I error: type I error to determine the LOD threshold from permutation tests.

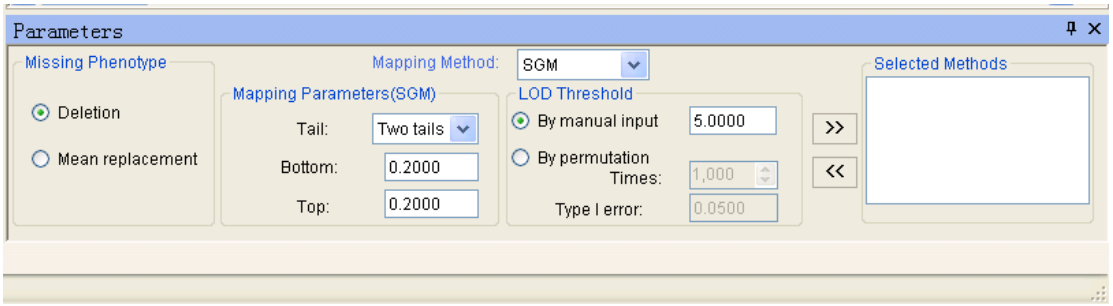


Figure 4.6 Parameters for SGM

4.4 Outputs

In QTL IciMapping, the output files have the same prefix name as the input file but with different extension names. Four output files record some general information of the mapping population. Each method (i.e. SMA, SIM, ICIM-ADD, ICIM-EPI or SGM) has three kinds of output, denoted as R (for results at any scanning position), Q (for significant QTL), and T (for permutation tests). Five additional files are given for ICIM-EPI in a lower triangular format. File LOD is determined only by the epistatic effects. File IAA, is available for ICIM-EPI in populations where two genotypes are present, such as DH or RIL. Files IAA, IAD, IDA, and IDD are available for ICIM-EPI in populations where three genotypes are present, such as F_2.

4.4.1 General information output files

1. STA file: Basic statistics of phenotypic data in the population (Table 4.6)

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - n: size of the mapping population.
   - Mean: Mean of the phenotypic trait.
   - Variance: Variance of the phenotypic trait.
   - Std: Standard deviation of the phenotypic trait.
   - Skewness: Skewness of the phenotypic trait.
   - Kurtosis: Kurtosis of the phenotypic trait.
   - Min: Minimum value of the phenotypic trait.
   - Max: Maximum value of the phenotypic trait.
   - Range: Range of the phenotypic trait.
   - W-test: The Shapiro Wilk W-statistic for the test of normality.
   - P-Value: P-value of the W-test of normality.

Table 4.6 Basic statistics of phenotypic data in the population (STA)

TraitID	TraitName	n	Mean	Variance	Std	Skewness	Kurtosis	Min	Max	Range	W-test	P-value
1	KWT	145	42.5029	4.9468	2.2241	0.1439	-0.1511	36.4516	48.4600	12.0084	0.9840	0.6686

2. COE file: Correlation coefficient matrix between markers (Table 4.7)

   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: Marker name, same as given in the BIP input file.
   - First row: Number after MarkerID and MarkerName is also Marker ID.
   - Others: Correlation coefficient between two markers.

Table 4.7 Correlation coefficient matrix between markers (COE, incomplete)

MarkerID	MarkerName	1	2	3	4	5	6	……
1	RM1-004	1.0000	0.4163	0.3906	0.2606	0.2142	0.1886	……

2	RM1201	0.4163	1.0000	0.9010	0.7055	0.5930	0.5268	……
3	RM490	0.3906	0.9010	1.0000	0.7651	0.6158	0.5379	……
4	RM576	0.2606	0.7055	0.7651	1.0000	0.7391	0.6094	……
5	RM600	0.2142	0.5930	0.6158	0.7391	1.0000	0.7795	……
6	RM562	0.1886	0.5268	0.5379	0.6094	0.7795	1.0000	……

……

3. MTP file: There are two parts in this output file. The first part contains information for each marker (Table 4.8), and the second part gives the marker types after imputation of missing markers (Table 4.9). Markers are in the order as in the linkage map.

   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: name of each marker
   - Chromosome: ID number starting from 1 for each chromosome
   - Position: the position of the current marker in the linkage group
   - n(AA): the number of genotypes same as the first parent (P1), which denoted as 2 in the input file.
   - n(Aa): the number of genotypes same as F1, which denoted as 1 in the input file.
   - n(aa): the number of genotypes same as the second parent (P2), which denoted as 0 in the input file.
   - n(-): the number of missing genotypes, which denoted as -1 in the input file.
   - Chi^2-Test: χ^2-test statistics testing for segregation distortion of markers.
   - P(x>Chi^2): the corresponding probability for the χ^2-test statistics, i.e., which is equal to P(x> Chi^2-Test).

Table 4.8 Marker information and test of segregation distortion (MTP Part 1, incomplete)

MarkerID	MarkerName	Chromosome	Position	n(AA)	n(Aa)	n(aa)	n(-)	Chi^2-Test	P(x>Chi^2)
1	RM1-004	1	0.0000	45	91	30	14	4.2530	0.1193
2	RM1201	1	35.8000	39	106	26	9	11.8070	0.0027
3	RM490	1	40.1000	39	87	25	29	6.0993	0.0474
4	RM576	1	50.4000	48	101	27	4	8.8523	0.0120
5	RM600	1	60.9000	44	92	22	22	10.4051	0.0055

……

Table 4.9 Marker types after imputation of missing markers (MTP Part 2, incomplete)

!**********Marker Type**********																						
!Marker type: 2 for P1; 1 for F1; 0 for P2; BC1=F1xP1; BC2=F1xP2; -1 for missing markers																						
RM1-004			1	0	0	2	1	2	2	2	1	1	0	1	1	0	0	1	2	0	2	
0	1	1	0	1	1	1	1	1	2	1	2	1	2	2	2	1	1	1	1	1	1	2	2	1
1	1	2	1	1	1	1	0	2	0	2	1	2	2	0	2	2	1	0	2	1	0	2	0	1
1	1	1	1	1	1	1	1	0	2	1	1	2	1	1	0	2	2	1	0	1	1	1	1	2
1	1	1	0	2	1	1	2	1	1	0	1	0	1	1	1	0	2	1	2	1	2	0	1	1
1	1	1	2	1	2	2	0	0	1	1	1	2	1	0	2	0	1	0	2	1	1	1	2	1
1	1	2	0	1	1	0	1	1	0	2	1	1	1	1	2	1	0	1	1	2	1	2	2	0
0	1	1	2	1	1	0	1	1	1	2												
RM1201			1	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	1	1	1	
0	1	1	1	2	1	1	1	1	1	2	2	1	1	2	1	2	1	1	1	0	2	1	0	2
2	1	1	1	1	2	2	0	2	2	2	1	2	1	1	1	1	1	1	1	1	2	1	1	1
0	2	1	1	2	1	1	0	1	1	1	1	2	0	1	0	1	2	2	1	2	1	0	1	2
1	1	1	1	2	1	1	1	1	2	0	1	0	0	0	1	1	2	1	1	1	1	0	1	2
1	1	2	1	0	2	2	0	1	0	1	0	2	1	1	2	1	1	1	2	1	1	1	2	1
1	2	1	1	0	1	0	1	1	0	2	1	1	1	2	2	1	1	1	1	1	1	2	2	0

```
1  1  1  2  2  1  0  1  2  1  2
RM490              1  1  1  1  1  1  1  0  1  1  0  1  1  0  0  1  1  1  1
0  1  1  1  2  1  1  1  1  1  1  2  1  2  1  1  2  1  1  1  0  1  2  0  2
2  1  1  1  1  2  2  0  2  2  2  1  2  1  1  1  0  1  1  1  1  2  1  1  1
0  2  1  1  2  1  1  0  1  1  1  1  2  2  1  0  1  2  2  1  2  1  0  1  2
1  1  1  1  2  1  1  1  2  2  0  1  0  0  0  1  1  2  1  1  1  1  0  1  2
1  1  2  1  0  2  2  0  1  0  1  0  2  1  1  2  2  1  1  2  1  1  1  2  1
1  2  1  1  0  0  0  1  1  0  2  1  2  1  2  2  1  1  1  1  1  1  2  2  0
1  1  1  2  2  1  0  1  2  1  2
……
```

4. STP file: Results from stepwise regression (Table 4.10)

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - Type: To distinct between the two stepwise regressions for additive and epsiatsis mapping.
   - Para: Parameter coefficient after the stepwise regression.
   - Numbers after Para are markers retained in the stepwise regression.
   - Intercept: Intercept of the stepwise regression.
   - R^2: Phenotypic variation explained by the final regression model. In QTL additive mapping, this can be viewed as the total phenotypic variation explained by all additive QTL. In QTL epistasis mapping, this can be viewed as the total phenotypic variation explained by all QTL interactions.

Table 4.10 Retained markers and their coefficients in stepwise regression (STP)

TraitID	TraitName	Type	Para	34	106	292	Intercept	R^2
1	Resistance	ADD	COEF	-1.1031	-0.9347	-1.1990	5.4201	19.1678

4.4.2 Results from all scanning markers or chromsomal positions

1. RSM file: Mapping results from single marker analysis for QTL with additive (and dominance) effects at any tesing positions (Table 4.11)

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: Marker name, same as given in the BIP input file.
   - Chromosome: Chromosome ID represented by an integer number.
   - Position: Marker position in cM on the chromosome.
   - LOD: LOD score calculated from single marker analysis.
   - PVE (%): Phenotypic variation expelained by the marker.
   - EstA: Estimated additive effect of the marker.
   - EstD: Estimated dominance effect of the marker.
   - M (QQ): Mean value of the QTL genotype QQ (the genotype of P1).
   - M (Qq): Mean value of the QTL genotype Qq (the genotype of F1).
   - M (qq): Mean value of the QTL genotype qq (the genotype of P2).

Table 4.11 Results of single marker analysis (RSM, incomplete)

TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	LOD	PVE(%)	EstA	EstD	M(QQ)	M(Qq)	M(qq)
1	Resistance	1	RM1-004	1	0.0000	0.1075	0.2745	-0.1934	-0.1527	4.5677	4.6084	4.9545
1	Resistance	2	RM1201	1	35.8000	0.1347	0.3441	-0.0645	0.300	4.4207	4.7857	4.5498
1	Resistance	3	RM490	1	40.1000	0.4367	1.1110	-0.4091	0.1612	4.2000	4.7703	5.0182

……

2. RIM and RAD files: Results from simple interval mapping and ICIM-ADD for QTL with additive (and dominance) effects at any tesing positions (Table 4.12)

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - Chromosome: Chromosome ID represented by an integer number.
   - Position: The scanning position in cM on the chromosome.
   - LOD: LOD score.
   - PVE (%): Phenotypic variation expelained by QTL at the current scanning position.
   - EstA: Estimated additive effect of QTL at the current scanning position.
   - EstD: Estimated dominance effect of QTL at the current scanning position.
   - M (QQ): Mean value of the QTL genotype QQ (the genotype of P1).
   - M (Qq): Mean value of the QTL genotype Qq (the genotype of F1).
   - M (qq): Mean value of the QTL genotype qq (the genotype of P2).

Table 4.12 Results of simple interval mapping and ICIM-ADD (RIM or RAD, incomplete)

Trait	TraitName	Chromosome	Position	LOD	PVE(%)	EstA	EstD	M(QQ)	M(Qq)	M(qq)
1	Resistance	1	0.0000	0.1075	0.2745	-0.1934	-0.1527	4.5677	4.6084	4.9545
1	Resistance	1	1.0000	0.2082	0.2832	-0.1987	-0.1485	4.5604	4.6106	4.9578
1	Resistance	1	2.0000	0.3034	0.2909	-0.2042	-0.1420	4.5517	4.6140	4.9601
1	Resistance	1	3.0000	0.3932	0.2975	-0.2098	-0.1324	4.5414	4.6187	4.9609

……

3. REP file: Results from two-dimensional scanning for QTL networks at any testing positions (Part 1 in Tables 4.13, Part 2 in Table 4.14, and Part 3 in Table 4.15)

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - Chromosome1: Chromosome ID at the first scanning position.
   - Position1: Scanning position in cM of the first QTL.
   - Chromosome2: Chromosome ID at the second scanning position.
   - Position2: Scanning position in cM of the second QTL.
   - LOD: LOD score caused by epistasis effects.
   - PVE: Phenotypic variation expelained by epsitatic QTL effects.

   - EstA1: Estimated additive effect of the first QTL.
   - EstA2: Estimated additive effect of the second QTL.
   - EstD1: Estimated dominance effect of the first QTL.
   - EstD2: Estimated dominance effect of the second QTL.
   - EstAA: Estimated additive by additive effect of QTL at the two scanning

positions.

- EstAD: Estimated additive by dominance effect of QTL at the two scanning positions.
- EstDA: Estimated dominance by additive effect of QTL at the two scanning positions.
- EstDD: Estimated dominance by dominance effect of QTL at the two scanning positions.

- M (Q1Q1Q2Q2): Mean value of the QTL genotype Q1Q1Q2Q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M (Q1Q1Q2q2): Mean value of the QTL genotype Q1Q1Q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M (Q1Q1q2q2): Mean value of the QTL genotype Q1Q1q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M (Q1q1Q2Q2): Mean value of the QTL genotype Q1q1Q2Q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M (Q1q1Q2q2): Mean value of the QTL genotype Q1q1Q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M (Q1q1q2q2): Mean value of the QTL genotype Q1q1q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M (q1q1Q2Q2): Mean value of the QTL genotype q1q1Q2Q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M (q1q1Q2q2): Mean value of the QTL genotype q1q1Q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M (q1q1q2q2): Mean value of the QTL genotype q1q1q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.

Table 4.13 Results of ICIM-EPI Part 1 (REP, incomplete)

TraitID	TraitName	Chromosome1	Position1	Chromosome2	Position2	LOD	PVE
……							
1	Resistance	1	0.0000	1	95.0000	0.7647	1.5832
1	Resistance	1	5.0000	1	95.0000	1.2839	5.6049
1	Resistance	1	10.0000	1	95.0000	1.8996	8.5647
……							

Table 4.14 Results of ICIM-EPI Part 2 (REP, incomplete)

EstA1	EstA2	EstD1	EstD2	EstAA	EstAD	EstDA	EstDD
……							
-0.5574	-0.1590	0.4095	0.8824	0.4594	0.6837	0.2898	-0.7370
-0.2918	-0.5824	1.3477	1.5398	1.0976	0.4979	0.9891	-1.6957
-0.3278	-0.6957	1.6252	1.6009	1.1816	0.7169	1.3218	-1.8729
……							

Table 4.15 Results of ICIM-EPI Part 3 (REP, incomplete)

M(Q1Q1Q2Q2)	M(Q1Q1Q2q2)	M(Q1Q1q2q2)	M(Q1q1Q2Q2)	M(Q1q1Q2q2)	M(Q1q1q2q2)	M(q1q1Q2Q2)	M(q1q1Q2q2)	M(q1q1q2q2)
……								
4.6455	5.9112	4.0447	5.4428	5.4575	5.1813	4.8415	5.6587	6.0784
4.4761	5.9986	3.4457	6.0072	5.4446	5.1938	2.8645	5.5865	6.2246
4.2814	6.1133	3.3097	6.3745	5.4764	5.1223	2.5737	5.3350	6.3284
……								

4. RSG file: Results from selective genotyping for QTL at any tesing positions (Table 4.16)

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: Marker name, same as given in the BIP input file.
   - Chromosome: Chromosome ID represented by an integer number.
   - Position: Marker position in cM on the chromosome.
   - LOD: LOD score calculated from selective genotyping.
   - FreqDiff: Difference in marker frequency in the two sub-populations in selective genotyping.
   - FreqTop: Marker frequency in the top sub-population in selective genotyping, if applicable.
   - FreqUnsel: Marker frequency in the original unselected population.
   - FreqBottom: Marker frequency in the bottom sub-population in selective genotyping, if applicable.

Table 4.16 Results of selective genotyping (RSG, incomplete)

TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	LOD	FreqDiff	FreqTop	FreqUnsel	FreqBottom
1	Resistance	1	RM1-004	1	0.0000	0.0241	-0.0278	0.4861	0.5000	0.5139
1	Resistance	2	RM1201	1	35.8000	0.0546	-0.0417	0.5139	0.5000	0.5556
1	Resistance	3	RM490	1	40.1000	0.3896	-0.1111	0.4861	0.5000	0.5972
……										

5. LOD file: LOD score tesing only the epistatic varaiation during the two-dimensional scanning (Table 4.17)

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - Chromosome: Chromosome ID at the first scanning position.
   - Position: Scanning position in cM of the first QTL.
   - Others: LOD score between the two scanning positions.

Table 4.17 LOD score during the two-dimensional scanning (LOD, incomplete)

TraitID	TraitName	Chromosome	Position	
1	Resistance	1	0.0000	
1	Resistance	1	5.0000	0.0000

```
      1        Resistance          1    10.0000     0.0000     0.0000
      1        Resistance          1    15.0000     0.0000     0.0000     0.0000
……
```

6. IAA, IAD, IDA, and IDD files: additive by additive effect, additive by dominance effect, dominance by additive effect, and dominance by dominance effect from the two-dimensional scanning of all populations. Format of these files is the similar to that shown in Table 4.17.

4.4.3 Results files for significant QTL

1. QSM file: All significant markers from single marker analysis (Table 4.18)

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: Marker name, same as given in the BIP input file.
   - Chromosome: Chromosome ID represented by an integer number.
   - Position: Marker position in cM on the chromosome.
   - LOD: LOD score calculated from single marker analysis.
   - PVE (%): Phenotypic variation expelained by the marker.
   - EstA: Estimated additive effect of the marker.
   - EstD: Estimated dominance effect of the marker.

Table 4.18 Significant markers from single marker analysis (QSM, incomplete)

TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	LOD	PVE(%)	EstA	EstD
1	Resistance	33	RM40	4	0.0000	3.1382	7.7152	-0.9505	0.6472
1	Resistance	34	RM335	4	7.3000	3.5792	8.7502	-1.1619	0.2077
1	Resistance	35	RM4_62	4	29.4000	3.1232	7.6794	-1.0165	-0.1089
1	Resistance	36	RM471	4	29.7000	2.9728	7.3237	-0.9921	-0.1709

2. QIM and QAD files: Significant QTL from simple interval mapping and ICIM-ADD (significant additive and dominance QTL can be selected from this file; Table 4.19).

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - Chromosome: Chromosome ID represented by an integer number.
   - Position: The scanning position in cM on the chromosome.
   - LeftMarker: Name of the left-side marker of the identified QTL.
   - RightMarker: Name of the right-side marker of the identified QTL.
   - LOD: LOD score.
   - PVE (%): Phenotypic variation expelained by QTL at the current scanning position.
   - EstA: Estimated additive effect of QTL at the current scanning position.
   - EstD: Estimated dominance effect of QTL at the current scanning position.

Table 4.19 Significant QTL from SIM or ICIM-ADD (QIM or QAD, incomplete)

```
Trait   TraitName  Chromosome  Position  LeftMarker  RightMarker    LOD   PVE(%)   EstA      EstD
    1   Resistance          4   17.0000      RM335       RM4_62  5.0996  14.8610  -1.4826   0.1010
……
```

3. QEP file: All significant peaks from two-dimensional scanning for QTL networks (significant digenic interacting QTL from ICIM can be selected from this file; Part 1 is in Table 4.20, and Part 2 is similar to Table 4.14).

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - Chromosome1: Chromosome ID at the first scanning position.
   - Position1: Scanning position in cM of the first QTL.
   - Chromosome2: Chromosome ID at the second scanning position.
   - Position2: Scanning position in cM of the second QTL.
   - LOD: LOD score caused by epistasis effects.
   - PVE: Phenotypic variation expelained by epsitatic QTL effects.
   - EstA1: Estimated additive effect of the first QTL.
   - EstA2: Estimated additive effect of the second QTL.
   - EstD1: Estimated dominance effect of the first QTL.
   - EstD2: Estimated dominance effect of the second QTL.
   - EstAA: Estimated additive by additive effect of QTL at the two scanning positions.
   - EstAD: Estimated additive by dominance effect of QTL at the two scanning positions.
   - EstDA: Estimated dominance by additive effect of QTL at the two scanning positions.
   - EstDD: Estimated dominance by dominance effect of QTL at the two scanning positions.

Table 4.20 Significant interactions from ICIM-EPI Part 1 (QEP, incomplete)

```
TraitID  TraitName Chr1    Pos1  LeftMarker1  RightMarker1 Chr2    Pos2 LeftMarker2 RightMarker2  LOD     PVE
     1  Resistance    3  15.0000       RM81 B       RM3467    10  20.0000       RM222     RM6646  5.3306  16.5849
     1  Resistance    6   5.0000        RM6_7       RM6_13    12  50.0000      RM6296     RM1246  5.0960  15.0973
……
```

4. QSG file: All significant peaks from selective genotyping for QTL (Table 4.21)

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: Marker name, same as given in the BIP input file.
   - Chromosome: Chromosome ID represented by an integer number.
   - Position: Marker position in cM on the chromosome.
   - LOD: LOD score calculated from selective genotyping.
   - FreqDiff: Difference in marker frequency in the two sub-populations in selective genotyping.

Table 4.21 Significant interactions from selective genotyping (QSG, incomplete)

TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	LOD	FreqDiff
1	Resistance	34	RM335	4	7.3000	2.7132	-0.2917
1	Resistance	35	RM4_62	4	29.4000	2.4468	-0.2778

5. GTP file: The posterior probability and predicted genotype for each identified QTL in each individual, and the predicted genotypic value for each individual (Table 4.22). This file is only for ICIM-ADD.

   - TraitID: Trait ID represented by an integer number.
   - TraitName: Trait name, same as given in the BIP input file.
   - Sample: Sample ID represented by an integer number.
   - Phenotype: Phenotypic value of the traits, as given in the input file.
   - P(QQ)_001: Posterior probability of QTL genotype QQ of the sample at the first identified QTL.
   - P(Qq)_001: Posterior probability of QTL genotype Qq of the sample at the first identified QTL.
   - P(qq)_001: Posterior probability of QTL genotype qq of the sample at the first identified QTL.
   - Genotype: QTL genotype of the sample determined by the posterior Bayesian probabilities.

Table 4.22 QTL genotype determined by Bayesian posterior probabilities (GTP, incomplete)

TraitID	TraitName	Sample	Phenotype	EstValue	P(QQ)_001	P(Qq)_001	P(qq)_001	Genotype ……
1	Resistance	1	7.9020	-0.1341	0.0120	0.9822	0.0059	Qq
1	Resistance	2	2.3000	-1.5196	0.5240	0.4435	0.0325	QQ
1	Resistance	3	6.7308	1.5196	0.0000	0.0243	0.9757	qq
……								

4.4.4 Results files from permutation tests

The output files from permutation tests for SMA, SIM, ICIM-ADD, ICIM-EPI and SGM have the extension names TSM, TIM, TAD, TEP and TSG, and the same format (Table 4.23).

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Times: Times in permutation tests. The threshold LOD was given after all permutation tests.
- MaxLOD: Maximum LOD score in the permutation test.

Table 4.23 Results of permutation tests for the five methods (TSM, TIM, TAD, TEP and TSG, incomplete)

TraitID	TraitName	Times	MaxLOD
1	Resistance	1	2.6697
1	Resistance	2	1.6576
……			
1	Resistance	10	2.0346
……			
1	Resistance	Threshold	2.9023
……			

4.5 Figures

One can also draw the linkage map defined in a BIP file. Please refer “3.8 Draw linkage maps” for details.

4.5.1 Figures from ICIM additive mapping (ICIM-ADD)

Click tool bar 

or ICIM for additive mapping in the Figures menu to draw graphs from the ICIM-ADD method. A new window with the title “[Graph] ICIM for additive mapping - …” will be open (Figures 4.7 and 4.8). Tool bars are available to change the figure format. Purposes of these tool bars are explained in Figure 4.8. Graphs can be shown one by one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 4.7).

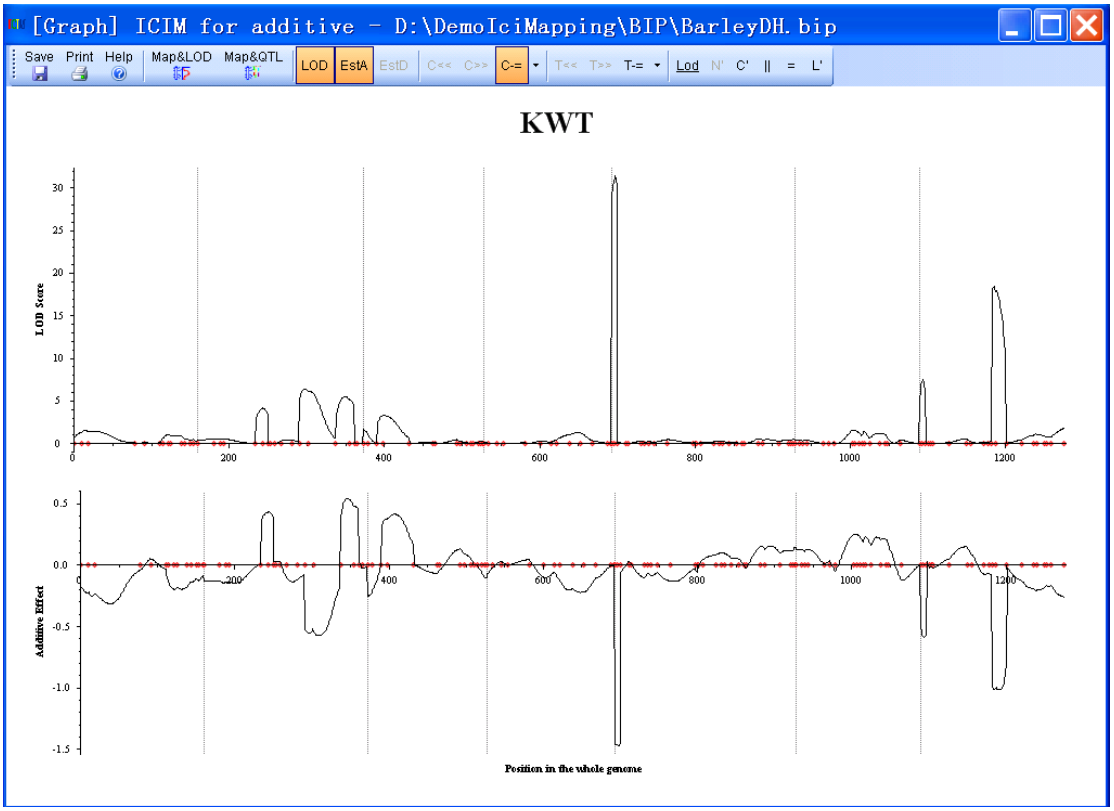


Figure 4.7 Figures from ICIM additive mapping (ICIM-ADD)

The software also provides figures to combine LOD score with the linkage map (Figure 4.9A), and identified QTL with linkage map (Figure 4.9B). As the graphs for the LOD score / estimated genetic effects, the combined figures can also be shown one by one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 4.9).

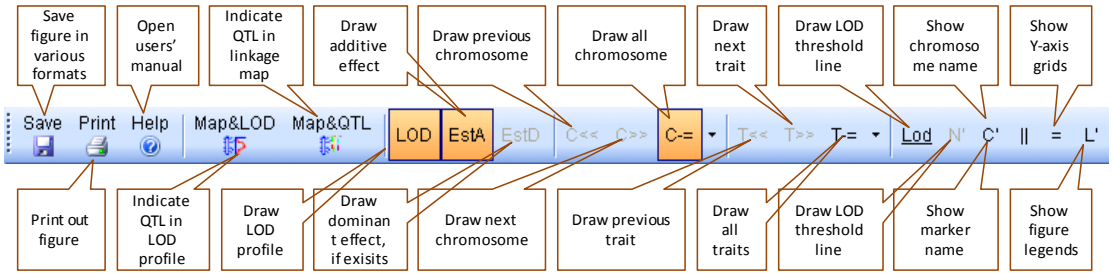
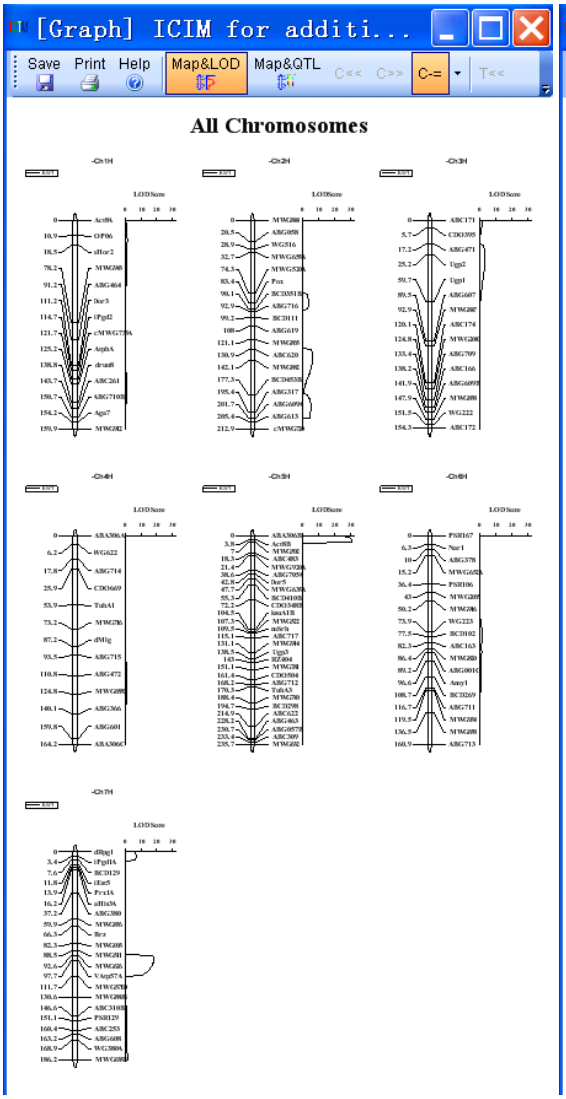


Figure 4.8 Tool bars in figures from ICIM additive mapping (ICIM-ADD)

A. Combined figure of linkage map and LOD score



B. Combined figure of linkage map and identified QTL

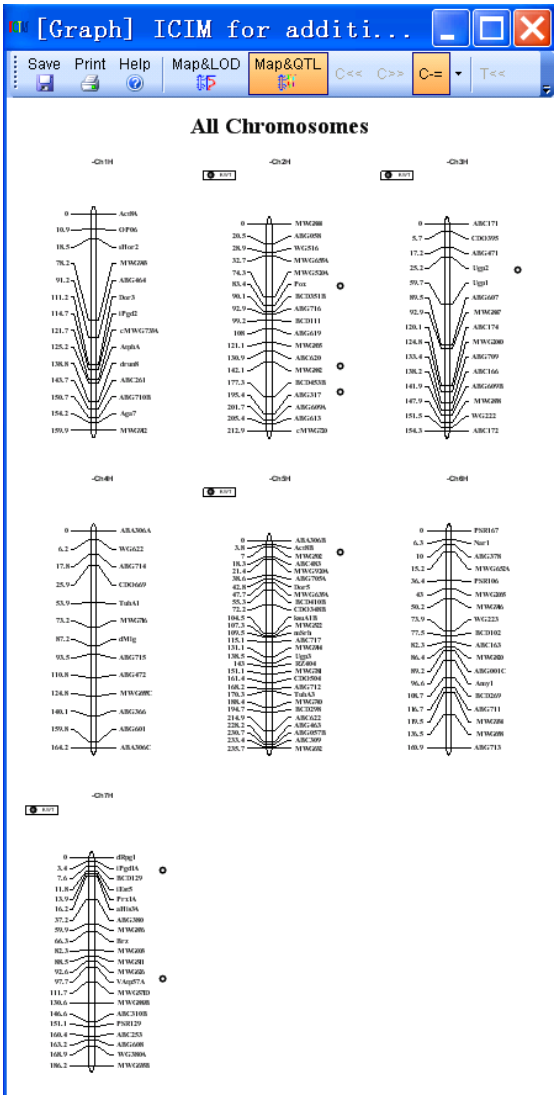


Figure 4.9 Combined Figure from ICIM additive mapping (ICIM-ADD)

4.5.2 Figures from ICIM epistatic mapping (ICIM-EPI)

Click tool bar 

or ICIM for epistatic mapping in the Figures menu to draw

graphs from the ICIM-EPI method. A new window with the title “[Graph] ICIM for epistatic - …” will be open (Figure 4.10). Tool bars are available to change the figure format. Graphs are shown for all chrosmomes.

4.5.3 Figures from simple interval mapping (SIM)

Click Simple interval mapping in the Figures menu to draw graphs from the SIM method. A new window with the title “[Graph] Simple interval mapping - …” will be open (Figure 4.11). Same tool bars as ICIM-ADD are available to change the figure format. Purposes of these tool bars are explained in Figure 4.8. Graphs can be shown one by one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 4.11).

4.5.4 Figures from single marker analysis (SMA)

Click Single marker analysis in the Figures menu to draw graphs from the SMA method. A new window with the title “[Graph] Single marker analysis - …” will be open to show the bar graph (Figure 4.12). Similar tool bars as ICIM-ADD are available to change the figure format. Graphs can be shown for markers on one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 4.12).

4.5.5 Figures from selective genotyping mapping (SGM)

Click Selective genotyping in the Figures menu to draw graphs from the SGM method. A new window with the title “[Graph] Selective genotyping - …” will be open to show the bar graph (Figure 4.13). Same tool bars as SMA are available to change the figure format. Graphs can be shown for markers on one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 4.13).

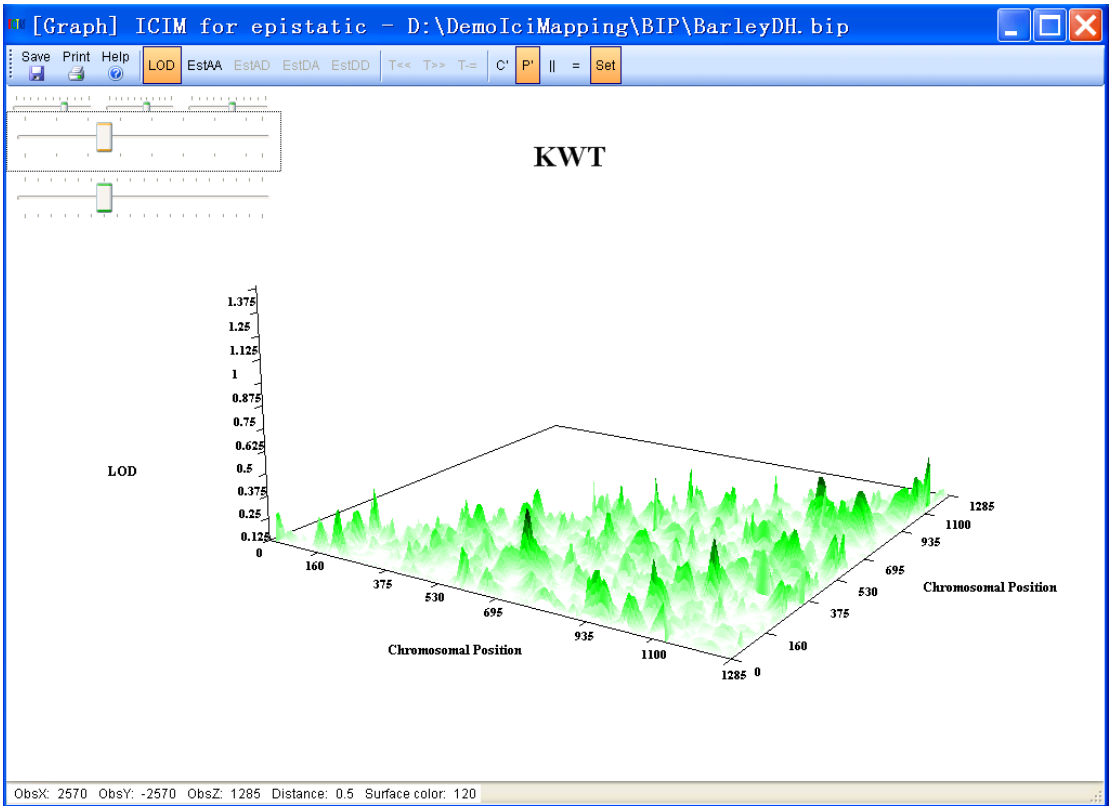


Figure 4.10 Figures from ICIM epistatic mapping (ICIM-EPI)

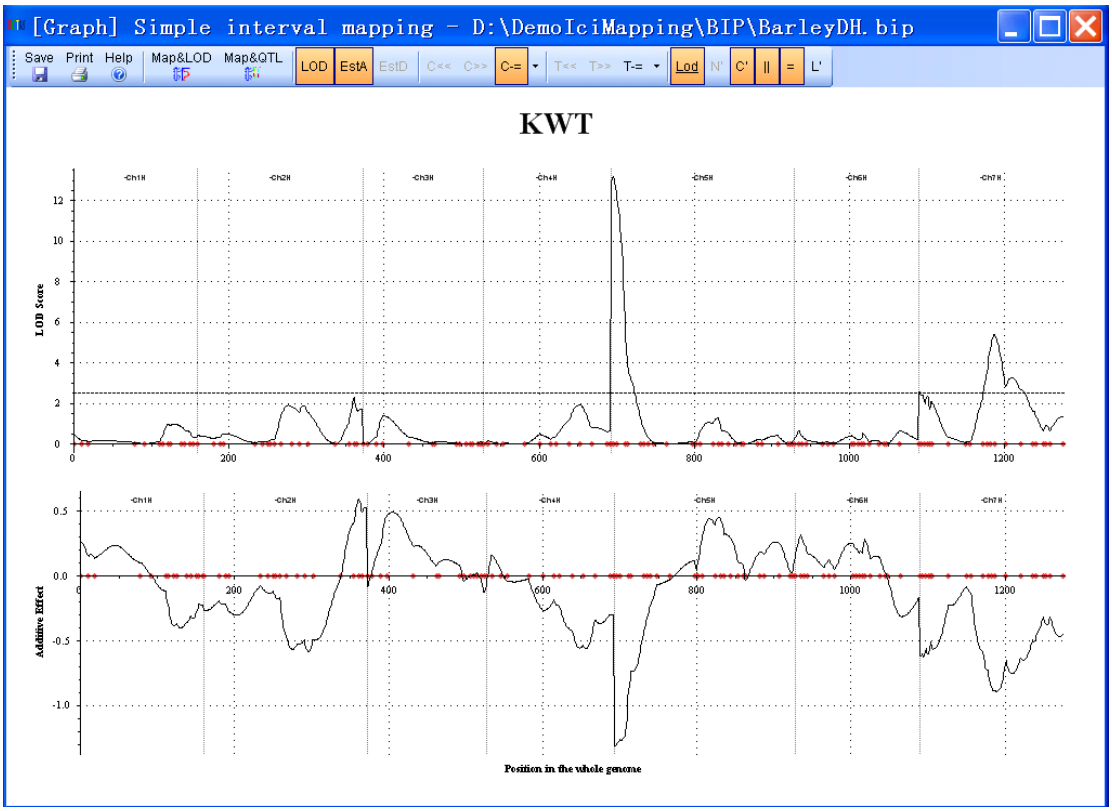


Figure 4.11 Figures from simple interval mapping (SIM)

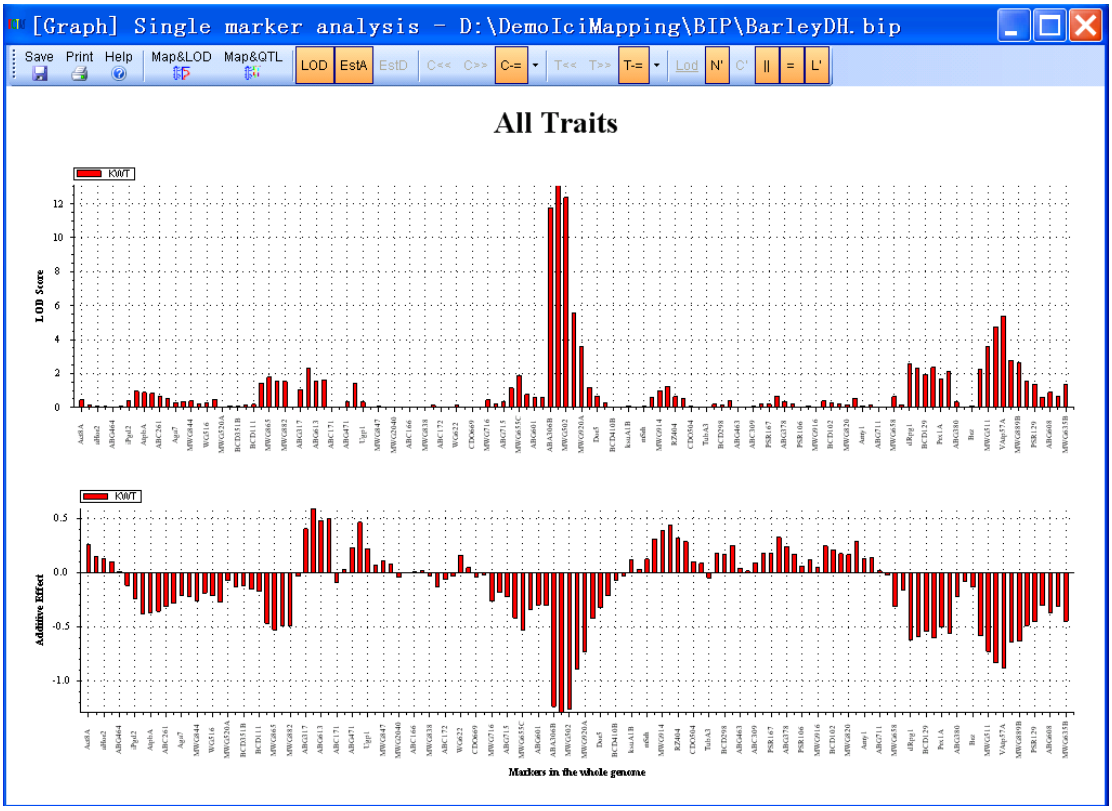


Figure 4.12 Figures from single marker analysis (SMA)

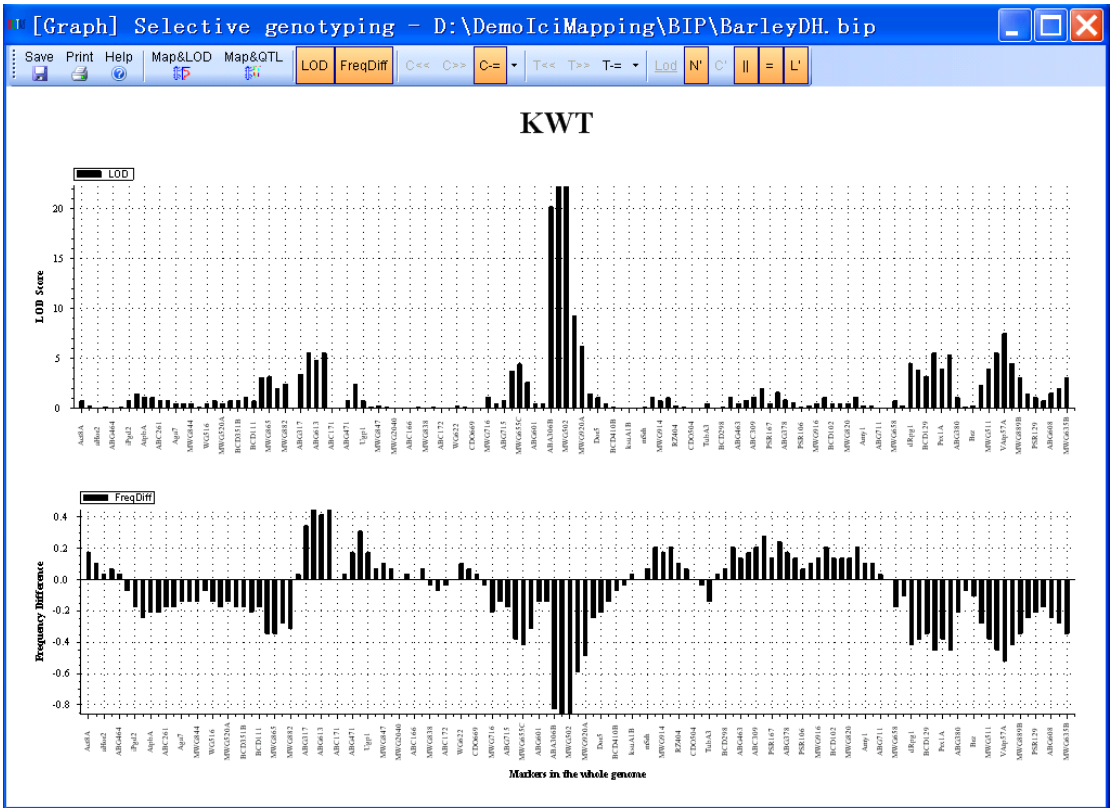


Figure 4.13 Figures from selective genotyping mapping (SGM)

Chapter 5. Power analysis in biparental populations (BIP)

By using QTL IciMapping, one can also conduct power analysis for a set of predefined QTL so as to compare the efficiency of different mapping methods. Tables 5.1-5.3 give a working example for a P1BC1F1 population with 200 individuals for power analysis.

5.1 Input file for power analysis in biparental populations (*.bip)

5.1.1 General population information of the mapping population

The general information in power analysis is similar to that in QTL mapping. Eight parameters were used for the general information describing a mapping population (Table 5.1).

- Indicator: This indicator lets IciMapping know if a mapping study or power simulation will be conducted. Indicator 2 has to be assigned for any power simulation studies.
  - 1 for mapping study.
  - 2 for power simulation.
- Population Type: describe the type of the population. At present, QTL IciMapping can conduct linkage map construction for twenty populations derived from two parental lines (Figure 1.1). Assuming F1 = P1 x P2, the 20 biparental populations are:
  - 1. P1BC1F1: the backcross population where the first parent (P1) is used as the recurrent.
  - 2. P2BC1F1: the backcross population where the second parent (P2) is used as the recurrent.
  - 3. F1DH: doubled haploids derived from F1.
  - 4. RIL: recombination inbred lines derived from repeated selfing since F1 generation.
  - 5. P1BC1RIL: recombination inbred lines derived from the backcross population where the first parent is used as the recurrent.
  - 6. P2BC1RIL: recombination inbred lines derived from the backcross population where the second parent is used as the recurrent.
  - 7. F2: the selfing generation of F1.
  - 8. F3: the selfing generation of F2.
  - 9. P1BC2F1: the second backcrossing where P1 is used as the recurrent parent.
  - 10. P2BC2F1: the second backcrossing where P2 is used as the recurrent parent.
  - 11. P1BC2RIL: recombination inred lines through the repeated selfing of P1BC2F1.

- 12. P2BC2RIL: recombination inred lines through the repeated selfing of P2BC2F1.
- 13. P1BC1F2: the selfing generation of P1BC1F1.
- 14. P2BC1F2: the selfing generation of P2BC1F1.
- 15. P1BC2F2: the selfing generation of P1BC2F1.
- 16. P2BC2F2: the selfing generation of P2BC2F1.
- 17. P1BC1DH: P1BC1F1-derived doubled haploids.
- 18. P2BC1DH: P2BC1F1-derived doubled haploids.
- 19. P1BC2DH: P1BC2F1-derived doubled haploids.
- 20. P2BC2DH: P2BC2F1-derived doubled haploids.

- Mapping Function: specify the mapping function which will be used to transfer recombination frequency to mapping distance, or from mapping distance to recombination frequency.
  - 1 for Kosombi mapping function.
  - 2 for Haldane mapping function.
  - 3 for Morgan mapping function.

- Marker Space Type: specify whether the markers on a chromosome (or linkage group) are defined by positions or marker intervals.
  - 1 for intervals, i.e. the number behind a marker is the distance of the marker to its next marker. 0 is normally given for the last marker on a chromosome or a linkage group.
  - 2 for positions, i.e. the number behind each marker is the position of the marker on the chromosome or the linkage group.

- Marker Space Unit: specify the unit used in marker linkage group.
  - 1 for centi-Morgan (cM).
  - 2 for Morgan (M). 1 M = 100 cM.

- Number of Chromosomes: specify the number of chromosomes (or linkage groups) in the mapping population.

- Population Size: number of individuals in the mapping population.

- Number of Traits: number of traits phenotyped in the mapping population.

Table 5.1 General population information in a power analysis input file.

```
!********Note: lines staring with "!" are remarks and will be ignored in the program************
!*************************** General Information ***************************************
!Assuming F1 = P1 x P2, populations available in QTL IciMapping are:
!  1, P1BC1F1 = P1 x F1, the first backcrossing where P1 is used as the recurrent parent;
!  2, P2BC1F1 = P2 x F1, the first backcrossing where P2 is used as the recurrent parent;
!  3, F1DH, F1-derived doubled haploids;
!  4, RIL or F1RIL, recombination inbred lines through the repeated selfing of F1;
!  5, P1BC1RIL, recombination inbred lines through the repeated selfing of P1BC1F1;
!  6, P2BC1RIL, recombination inbred lines through the repeated selfing of P2BC1F1;
!  7, F2, the selfing generation of F1;
!  8, F3, the selfing generation of F2;
!  9, P1BC2F1, the second backcrossing where P1 is used as the recurrent parent;
! 10, P2BC2F1, the second backcrossing where P2 is used as the recurrent parent;
```

```
! 11, P1BC2RIL, recombination inbred lines through the repeated selfing of P1BC2F1;
! 12, P2BC2RIL, recombination inbred lines through the repeated selfing of P2BC2F1;
! 13, P1BC1F2, the selfing generation of P1BC1F1;
! 14, P2BC1F2, the selfing generation of P2BC1F1;
! 15, P1BC2F2, the selfing generation of P1BC2F1;
! 16, P2BC2F2, the selfing generation of P2BC2F1;
! 17, P1BC1DH, P1BC1F1-derived doubled haploids;
! 18, P2BC1DH, P2BC1F1-derived doubled haploids;
! 19, P1BC2DH, P1BC2F1-derived doubled haploids;
! 20, P2BC2DH, P2BC2F1-derived doubled haploids;
  2      !Indictor (1 for mapping, 2 for simulation)
  1      !Mapping Population Type (see remarks above)
  2      !Mapping Function (1 for Kosambi; 2 for Haldane; 3 for Morgan)
  2      !Marker Space Type (1 for intervals; 2 for positions)
  1      !Marker Space Unit(1 for centiMorgan; 2 for Morgan)
  5      !Number of Chromosomes (or Linkage Group)
200      !Population size of the mapping population
  4      !Number of traits
```

5.1.2 Linkage group information or chromosome information

The linkage group information in power analysis is also similar to that in QTL mapping, as shown in Table 5.2. The name of each chromosome and the number of markers on the chromosome were specified first (Table 5.2), followed by the definition of each chromosome (Table 5.3).

Table 5.2 Linkage group information in a power analysis input file.

```
!***********************Information for Chromosomes and Markers************************
!Chromosome     NumMarkers in each chromosome
 -Ch1           11
 -Ch2           11
 -Ch3           11
 -Ch4           11
 -Ch5           11
```

Table 5.3 Definition of each chromosome.

```
!Linkage map (Marker name followed by position or the interval length)
MK-1-1       1           0.00000
MK-1-2       1          10.00000
MK-1-3       1          20.00000
MK-1-4       1          30.00000
MK-1-5       1          40.00000
MK-1-6       1          50.00000
MK-1-7       1          60.00000
MK-1-8       1          70.00000
MK-1-9       1          80.00000
MK-1-10      1          90.00000
MK-1-11      1         100.00000
MK-2-1       2           0.00000
MK-2-2       2          10.00000
MK-2-3       2          20.00000
MK-2-4       2          30.00000
MK-2-5       2          40.00000
MK-2-6       2          50.00000
MK-2-7       2          60.00000
MK-2-8       2          70.00000
MK-2-9       2          80.00000
MK-2-10      2          90.00000
MK-2-11      2         100.00000
MK-3-1       3           0.00000
MK-3-2       3          10.00000
MK-3-3       3          20.00000
MK-3-4       3          30.00000
MK-3-5       3          40.00000
MK-3-6       3          50.00000
MK-3-7       3          60.00000
MK-3-8       3          70.00000
```

```
MK-3-9       3          80.00000
MK-3-10      3          90.00000
MK-3-11      3         100.00000
MK-4-1       4           0.00000
MK-4-2       4          10.00000
MK-4-3       4          20.00000
MK-4-4       4          30.00000
MK-4-5       4          40.00000
MK-4-6       4          50.00000
MK-4-7       4          60.00000
MK-4-8       4          70.00000
MK-4-9       4          80.00000
MK-4-10      4          90.00000
MK-4-11      4         100.00000
MK-5-1       5           0.00000
MK-5-2       5          10.00000
MK-5-3       5          20.00000
MK-5-4       5          30.00000
MK-5-5       5          40.00000
MK-5-6       5          50.00000
MK-5-7       5          60.00000
MK-5-8       5          70.00000
MK-5-9       5          80.00000
MK-5-10      5          90.00000
MK-5-11      5         100.00000
```

5.1.3 QTL information

A number of QTL for one or multiple traits have to be defined before conducting any power simulation studies. Table 5.4 contains four traits, and 3 QTL were defined for each trait. For each trait, the number of QTL on each chromosome has to be specified first. 0 was given for those chromosomes with no QTL. Then the positions of those QTL on the chromosomes were followed.

A lower triangular matrix was used to define the additive and additive by additive epistatic effects of all QTL for the trait of interest. For example, a 3×3 lower triangular matrix was defined for trait 1 (Table 5.4).

The heritability in the broad sense or error variance has to be specified to determine the phenotypic value of each individual in the mapping population. For trait 1, 0.6 was defined as the heritability as an indicator 1 was specified (Table 5.4).

Then, the mean of simulated phenotype corresponding to each trait needs to be specified.

Table 5.4 QTL information in a power analysis input file.

```
!QTL for trait 1
!Number of QTL, positions of all QTL on the chromosome
!A n by n (n is the number of total QTL) triangular matrix was used to define the QTL addtive
and digenic epistasis
1     35.00
1     53.00
1     22.00
0
0

   1.0000
   0.0000    1.2500
   0.0000    0.0000    1.5000

1          !Indicator: 1 for heritability, 2 for error variance
```

```
0.6         !Heritability, or error variance based on the above indicator
10.00       !Overall mean of the phenotypic value


!QTL for trait 2
!Number of QTL, positions of all QTL on the chromosome
!A n by n (n is the number of total QTL) triangular matrix was used to define the QTL addtive
and digenic epistasis
2     35.00   70.00
1     53.00
0
0
0

   1.0000
   0.0000    1.2500
   0.0000    0.0000    1.5000

1          !Indicator: 1 for heritabilty, 2 for error variance
0.6         !Heritability, or error variance based on the above indicator
10.00       !Overall mean of the phenotypic value


!QTL for trait 3
!Number of QTL, positions of all QTL on the chromosome
!A n by n (n is the number of total QTL) triangular matrix was used to define the QTL addtive
and digenic epistasis
1     35.00
1     53.00
1     22.00
0
0

   1.0000
   2.0000    1.2500
   0.0000    0.0000    1.5000

1          !Indicator: 1 for heritabilty, 2 for error variance
0.7         !Heritability, or error variance based on the above indicator
10.00       !Overall mean of the phenotypic value

!QTL for trait 4
!Number of QTL, positions of all QTL on the chromosome
!A n by n (n is the number of total QTL) triangular matrix was used to define the QTL addtive
and digenic epistasis
1     35.00
1     53.00
1     22.00
0
0

   0.0000
   2.0000    0.0000
   0.0000    0.0000    1.5000

1          !Indicator: 1 for heritabilty, 2 for error variance
0.7         !Heritability, or error variance based on the above indicator
     !Overall mean of the phenotypic value
```

5.2 Input file for power analysis in biparental populations (*.xls or *.xlsx)

One mapping population for power analysis in biparental populations can also be defined in an Excel file with the extension name ‘xls’ or ‘xlsx’. The file should be composed of three sheets: ‘GeneralInfo’ (similar to Table 5.1), ‘Chromosome’ (similar to Table 5.2), ‘LinkageMap’ (similar to Table 5.3), and ‘QTLInfo’ (similar to Table 5.4).

5.3 Setting mapping parameters

The BIP simulation functionality can be initiated by (1) opening input files, or (2) double clicking BIP files listed in the project window, or (3) clicking one BIP file in the display window. When the BIP simulation functionality is activated, the Display window shows the contents of the current input file, and the Parameter window is for interaction with users (Figure 5.1).

Five mapping methods can be conducted in IciMapping, i.e. single marker analysis (SMA), the traditional inyterval mapping (SIM), ICIM for QTL with additive(and dominance) effects (or one dimensional scanning) (ICIM-ADD), ICIM for digenic QTL networks (or two dimensional scanning) (ICIM-EPI), and selective genotyping mapping (SGM). The threshold LOD score declaring significant additive QTL or QTL networks has to be specified. Once parameters are set for a method, to click  to select the defined method. to click  to unselect a method in the Selected Methods box (Figure 5.1).

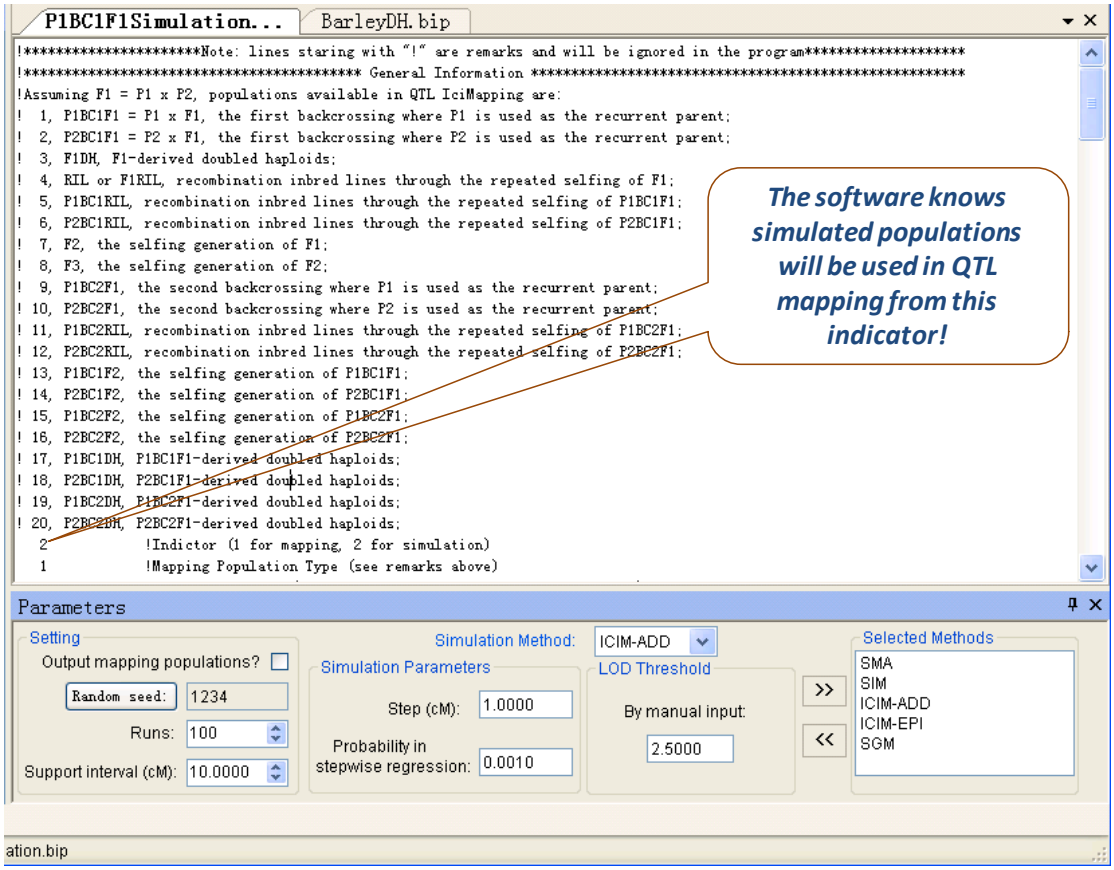


Figure 5.1 Display and Parameter windows in BIP simulation functionality

5.3.1 General information

Four parameters were defined as the general information for the five methods (Figure 5.1).

- Output mapping population indicator.
  - If selected: each simulated population will be output with the extension name 'bip', which is exactly the BIP format for QTL IciMapping.
  - Otherwise: no output of simulated populations.

- Random seed: initial seed for generating random numbers. Click Random seed: to change the random seed.
- Runs: number of simulation runs to be conducted.
- Support interval (cM): The length of the intervals of predefined QTL used in determining QTL detection powers. QTL is supposed at the center of the confidence interval. If one QTL is identified in the support interval of a predefined QTL, the predefined QTL is declared to be found.

5.3.2 Parmeters for SMA (Single Marker Analysis, Figure 5.2)

- LOD Threshold: the threshold LOD score to declare significant QTL.

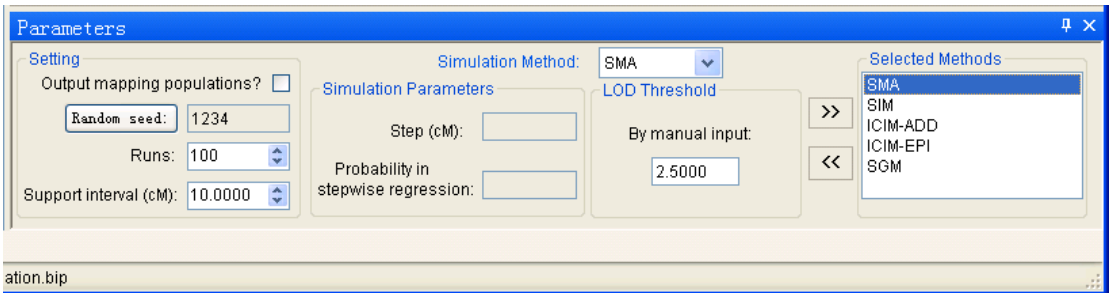


Figure 5.2 Parameters for SMA in BIP simulation

5.3.3 Parameters for SIM (Simple Interval Mapping, Figure 5.3)

- Step (cM): the step in scanning represented by cM.
- LOD Threshold: the threshold LOD score to declare significant QTL.

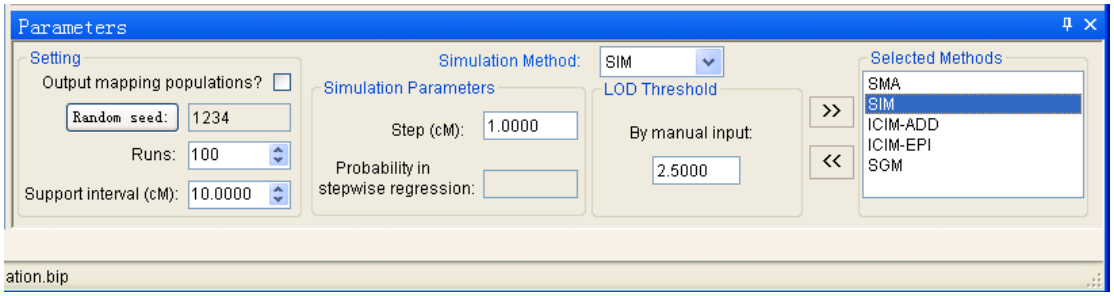


Figure 5.3 Parameters for SIM in BIP simulation

5.3.4 Parameters for ICIM of QTL with additive (and dominance) effects or one dimensional ICIM (Abbreviated as ICIM-ADD, Figure 5.4)

- Step (cM): the step in scanning represented by cM.
- Probability in stepwise regression: the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables (PIN). The largest P-value for removing variables is assumed to be two times of PIN.
- LOD Threshold: the threshold LOD score to declare significant QTL.

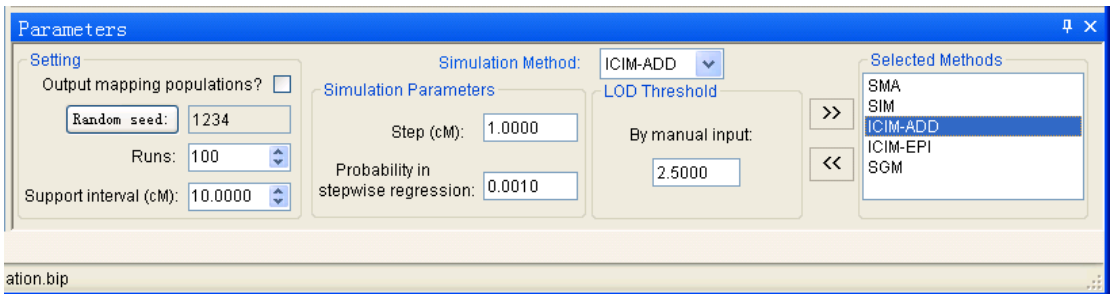


Figure 5.4 Parameters for ICIM-ADD in BIP simulation

5.3.5 Parameters for ICIM of digenic QTL networks or two dimensional ICIM (Abbreviated as ICIM-EPI, Figure 5.5)

- Step (cM): the step in scanning represented by cM.
- Probability in stepwise regression: the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables (PIN). The largest P-value for removing variables is assumed to be two times of PIN.
- LOD Threshold: the threshold LOD score to declare significant QTL.

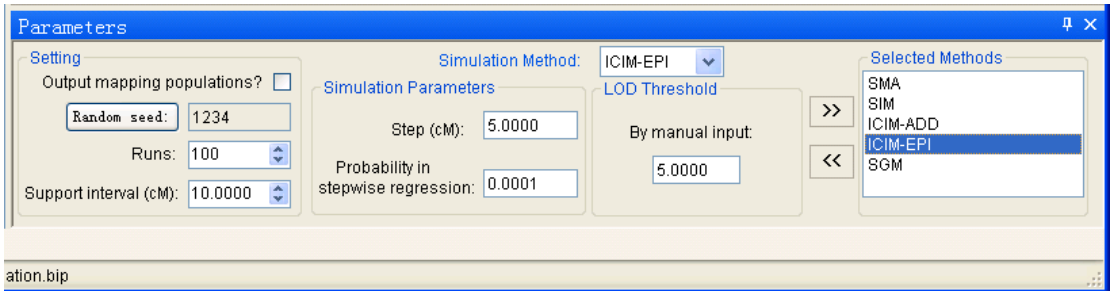


Figure 5.5 Parameters for ICIM-EPI in BIP simulation

5.3.6 Parameters for SGM (Selective Genotyping Mapping, Figure 5.6)

- Tail: conducting SGM using the top tail, the bottom tail, or both tails.
- Bottom: proportion of the bottom tail.
- Top: proportion of the top tail.
- LOD Threshold: the threshold LOD score to declare significant QTL.

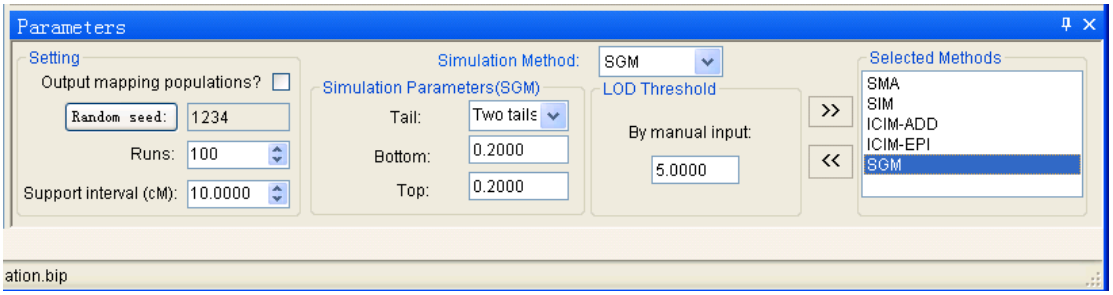


Figure 5.6 Parameters for SGM in BIP simulation

5.4 Outputs

Two general output files record some general information of the mapping population. Each method (i.e. SMA, SIM, ICIM-ADD, ICIM-EPI or SGM) has three kinds of output, denoted as R (for results), Q (for significant QTL), and P (for power simulation). Five additional files are given for ICIM-EPI in a lower triangular format. File LOD is determined only by the epistatic effects. File IAA, is available for ICIM-EPI in populations where two genotypes are present, such as DH or RIL. Files IAA, IAD, IDA, and IDD are available for ICIM-EPI in populations where three genotypes are present, such as F_2.

5.4.1 General files

1. STA file: Theoretical genetic variance and theoretical marker coefficients of the predefined QTL model (Part 1 in Table 5.5, Part 2 in Table 5.6, and Part 3 in Table 5.7)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Va: additive variance of the trait.
- Vi: epistatic variance of the trait.
- Vg: genetic variance of the trait.
- Va/Vg: the ratio of additive and genetic variance.
- Vi/Vg: the ratio of epistatic and genetic variance.
- Verror: the residual error variance.

- QTLID: QTL ID represented by an integer number.
- Chromosome: Chromosome ID represented by an integer number.
- Position: Marker position in cM on the chromosome.
- Effect (a): additive effect of QTL.
- LeftMarker: ID of the left-side marker of QTL.
- RightMarker: ID of the right-side marker of QTL.
- LeftPos: Scanning position in cM of the first QTL.
- RightPos: Scanning position in cM of the second QTL.
- LeftCoef: Theoretical coefficient of the first QTL.
- RightCoef: Theoretical coefficient of the second QTL.

- NetworkID: ID of network in epistatic mapping.
- Effect (aa): additive by additive effect of epistatic QTL.
- FirstQTL: ID of the first QTL in the network.
- Second QTL: ID of the second QTL in the network.
- L1xL2ID: ID of the interaction parameter for respective QTL (L for left marker of QTL, and R for right marker of QTL) in regression model.
- L1xR2ID: ID of the interaction parameter for respective QTL (L for left marker of QTL, and R for right marker of QTL) in regression model.
- R1xL2ID: ID of the interaction parameter for respective QTL (L for left marker of QTL, and R for right marker of QTL) in regression model.
- R1xR2ID: ID of the interaction parameter for respective QTL (L for left marker of QTL, and R for right marker of QTL) in regression model.
- L1xL2Coef: Coefficient of the interaction parameter for respective QTL (L for left marker of QTL, and R for right marker of QTL) in regression model.
- L1xR2Coef: Coefficient of the interaction parameter for respective QTL (L for left marker of QTL, and R for right marker of QTL) in regression model.
- R1xL2Coef: Coefficient of the interaction parameter for respective QTL (L for left marker of QTL, and R for right marker of QTL) in regression model.
- R1xR2Coef: Coefficient of the interaction parameter for respective QTL (L for left marker of QTL, and R for right marker of QTL) in regression model.

Table 5.5 Theoretical genetic variance of the predefined QTL model Part 1 (STA, incomplete)

TraitID	TraitName	Va	Vi	Vg(=Va+Vi)	Va/Vg	Vi/Vg	Verror
1	Trait1	4.8125	0.0000	4.8125	1.0000	0.0000	3.2083
2	Trait2	6.0540	0.0000	6.0540	1.0000	0.0000	4.0360
3	Trait3	4.8125	4.0000	8.8125	0.5461	0.4539	3.7768
4	Trait4	2.2500	4.0000	6.2500	0.3600	0.6400	2.6786

……

Table 5.6 Theoretical marker coefficients of the predefined QTL model Part 2 (STA,

incomplete)

TraitID	TraitName	QTLID	Chromosome	Position	Effect(a)	LeftMarker	RightMarker	LeftPos	RightPos	LeftCoef	RightCoef
1	Trait1	1	1	35.0000	1.0000	4	5	30.0000	40.0000	0.4975	0.4975
1	Trait1	2	2	53.0000	1.2500	17	18	50.0000	60.0000	0.8720	0.3727
1	Trait1	3	3	22.0000	1.5000	25	26	20.0000	30.0000	1.1971	0.2981

……

Table 5.7 Theoretical marker coefficients of the predefined QTL model Part 3 (STA, incomplete)

TraitID	TraitName	NetworkID	Effect(aa)	FirstQTL	SecondQTL	L1xL2ID	L1xR2ID	R1xL2ID	R1xR2ID	L1xL2Coef	L1xR2Coef	R1xL2Coef	R1xR2Coef
4	Trait4	1	2.0000	1	2	282	283	332	333	0.6942	0.2967	0.6942	0.2967

……

2. STP file: Results from stepwise regression for each simulation (Table 5.8)

- Simulation: Simulation ID represented by an integer number.
- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Type: To distinct between the two stepwise regressions for additive and epsiatsis mapping.
- Para: Parameter coefficient after the stepwise regression.
- Numbers after Para are markers retained in the stepwise regression.
- Intercept: Intercept of the stepwise regression.
- R^2: Phenotypic variation explained by the final regression model. In QTL additive mapping, this can be viewed as the total phenotypic variation explained by all additive QTL. In QTL espistasis mapping, this can be viewed as the total phenotypic variation explained by all QTL interactions.

Table 5.8 Retained markers and their coefficients in stepwise regression (STP, incomplete)

Simulation	TraitID	TraitName	Type	Para	18	25	Intercept	R^2
1	1	Trait1	ADD	COEF	0.7169	0.8311	11.9610	26.1891

……

Simulation	TraitID	TraitName	Type	Para	Intercept	R^2
1	1	Trait1	EPI	COEF	11.9610	0.0000

……

5.4.2 Results files from power analysis

1. RSM file: Average mapping results from single marker analysis for QTL with additive (and dominance) effects at any tesing positions (Table 5.9)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- MarkerID: Marker ID represented by an integer number.
- MarkerName: Marker name, same as given in the BIP input file.
- Chromosome: Chromosome ID represented by an integer number.
- Position: Marker position in cM on the chromosome.
- LOD: LOD score calculated from single marker analysis.
- PVE(%): Phenotypic variation expelained by the marker.

- EstA: Estimated additive effect of the marker.
- EstD: Estimated dominance effect of the marker.
- M(QQ): Mean value of the QTL genotype QQ (the genotype of P1).
- M(Qq): Mean value of the QTL genotype Qq (the genotype of F1).
- M(qq): Mean value of the QTL genotype qq (the genotype of P2).

Table 5.9 Results of single marker analysis (RSM, incomplete)

TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	LOD	PVE(%)	EstA	M(QQ)	M(Qq)
1	Trait1	1	MK-1-1	1	0.0000	0.2077	0.4770	0.2887	12.1706	11.8819
1	Trait1	2	MK-1-2	1	10.0000	0.4758	1.0898	0.4348	12.2443	11.8095
1	Trait1	3	MK-1-3	1	20.0000	0.4622	1.0586	0.4289	12.2394	11.8105
1	Trait1	4	MK-1-4	1	30.0000	0.9792	2.2291	0.6265	12.3115	11.6850
1	Trait1	5	MK-1-5	1	40.0000	0.6929	1.5827	0.5265	12.2738	11.7472
……										

2. RIM and RAD files: Average results from simple interval mapping and ICIM-ADD for QTL with additive (and dominance effects at any tesing positions (Table 5.10).

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Chromosome: Chromosome ID represented by an integer number.
- Position: The scanning position in cM on the chromosome.
- LOD: LOD score.
- PVE(%): Phenotypic variation expelained by QTL at the current scanning position.
- EstA: Estimated additive effect of QTL at the current scanning position.
- EstD: Estimated dominance effect of QTL at the current scanning position.
- M(QQ): Mean value of the QTL genotype QQ (the genotype of P1).
- M(Qq): Mean value of the QTL genotype Qq (the genotype of F1).
- M(qq): Mean value of the QTL genotype qq (the genotype of P2).

Table 5.10 Results of simple interval mapping and ICIM-ADD (RIM or RAD, incomplete)

TraitID	TraitName	Chromosome	Position	LOD	PVE(%)	EstA	M(QQ)	M(Qq)…
1	Trait1	1	0.0000	0.2077	0.4771	0.2887	12.1706	11.8819…
1	Trait1	1	1.0000	0.2376	0.5682	0.3150	12.1829	11.8680…
1	Trait1	1	2.0000	0.2689	0.6629	0.3400	12.1949	11.8548…
1	Trait1	1	3.0000	0.3005	0.7567	0.3632	12.2060	11.8428…
1	Trait1	1	4.0000	0.3317	0.8458	0.3838	12.2161	11.8323…
1	Trait1	1	5.0000	0.3620	0.9239	0.4010	12.2247	11.8237…
……								

3. REP file: Average results from two-dimensional scanning for QTL networks at any testing positions (Part 1 in Tables 5.11, Part 2 in Table 5.12, and Part 3 in Table 5.13)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Chromosome1: Chromosome ID at the first scanning position.
- Position1: Scanning position in cM of the first QTL.
- Chromosome2: Chromosome ID at the second scanning position.
- Position2: Scanning position in cM of the second QTL.

- LOD: LOD score caused by epistasis effects.
- PVE: Phenotypic variation expelained by epsitatic QTL effects.

- EstA1: Estimated additive effect of the first QTL.
- EstA2: Estimated additive effect of the second QTL.
- EstD1: Estimated dominance effect of the first QTL.
- EstD2: Estimated dominance effect of the second QTL.
- EstAA: Estimated additive by additive effect of QTL at the two scanning positions.
- EstAD: Estimated additive by dominance effect of QTL at the two scanning positions.
- EstDA: Estimated dominance by additive effect of QTL at the two scanning positions.
- EstDD: Estimated dominance by dominance effect of QTL at the two scanning positions.

- M(Q1Q1Q2Q2): Mean value of the QTL genotype Q1Q1Q2Q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M(Q1Q1Q2q2): Mean value of the QTL genotype Q1Q1Q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M(Q1Q1q2q2): Mean value of the QTL genotype Q1Q1q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M(Q1q1Q2Q2): Mean value of the QTL genotype Q1q1Q2Q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M(Q1q1Q2q2): Mean value of the QTL genotype Q1q1Q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M(Q1q1q2q2): Mean value of the QTL genotype Q1q1q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M(q1q1Q2Q2): Mean value of the QTL genotype q1q1Q2Q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M(q1q1Q2q2): Mean value of the QTL genotype q1q1Q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second scanning position. Q is from P1, and q is from P2.
- M(q1q1q2q2): Mean value of the QTL genotype q1q1q2q2. Q1 and q1 are the two alleles at the first scanning position, and Q2 and q2 are the two alleles at the second

scanning position. Q is from P1, and q is from P2.

Table 5.11 Results of ICIM-EPI Part 1 (REP, incomplete)

TraitID	TraitName	Chromosome1	Position1	Chromosome2	Position2	LOD	PVE …
1	Trait1	1	0.0000	1	5.0000	0.0000	0.0000 …
1	Trait1	1	0.0000	1	10.0000	0.0000	0.0000 …
1	Trait1	1	5.0000	1	10.0000	0.0000	0.0000 …
1	Trait1	1	0.0000	1	15.0000	0.0000	0.0000 …
1	Trait1	1	5.0000	1	15.0000	0.0000	0.0000 …
1	Trait1	1	10.0000	1	15.0000	0.0000	0.0000 …
1	Trait1	1	0.0000	1	20.0000	0.0000	0.0000 …
1	Trait1	1	5.0000	1	20.0000	0.0000	0.0000 …
1	Trait1	1	10.0000	1	20.0000	0.0000	0.0000 …
1	Trait1	1	15.0000	1	20.0000	0.0000	0.0000 …
1	Trait1	1	0.0000	1	25.0000	0.1876	0.3529 …
1	Trait1	1	5.0000	1	25.0000	0.1902	0.4847 …
1	Trait1	1	10.0000	1	25.0000	0.2015	0.4219 …
1	Trait1	1	15.0000	1	25.0000	0.0000	0.0000 …
1	Trait1	1	20.0000	1	25.0000	0.0000	0.0000 …

Table 5.12 Results of ICIM-EPI Part 2 (REP, incomplete)

… EstA1	EstA2	EstAA …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …
… -0.0908	0.6713	0.0130 …
… -0.1270	0.7089	0.0081 …
… -0.1172	0.7400	-0.0218 …
… 0.0000	0.0000	0.0000 …
… 0.0000	0.0000	0.0000 …

Table 5.13 Results of ICIM-EPI Part 3 (REP, incomplete)

… M(Q1Q1Q2Q2)	M(Q1Q1Q2q2)	M(Q1q1Q2Q2)	M(Q1q1Q2q2)
… 11.8570	11.8570	11.8570	11.8570
… 11.8570	11.8570	11.8570	11.8570
… 11.8570	11.8570	11.8570	11.8570
… 11.8565	11.8565	11.8565	11.8565
… 11.8565	11.8565	11.8565	11.8565
… 11.8565	11.8565	11.8565	11.8565
… 11.8565	11.8565	11.8565	11.8565
… 11.8565	11.8565	11.8565	11.8565
… 11.8565	11.8565	11.8565	11.8565
… 11.8568	11.8568	11.8568	11.8568
… 12.1535	11.4692	12.2313	11.5600
… 12.1503	11.4332	12.2692	11.5603
… 12.1532	11.4350	12.2922	11.5522
… 11.8494	11.8494	11.8494	11.8494
… 11.8494	11.8494	11.8494	11.8494

4. RSG file: Average results from selective genotyping for QTL at any tesing positions (Table 5.14)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- MarkerID: Marker ID represented by an integer number.
- MarkerName: Marker name, same as given in the BIP input file.

- Chromosome: Chromosome ID represented by an integer number.
- Position: Marker position in cM on the chromosome.
- LOD: LOD score calculated from selective genotyping.
- FreqDiff: Difference in marker frequency in the two sub-populations in selective genotyping.
- FreqTop: Marker frequency in the top sub-population in selective genotyping, if applicable.
- FreqUnsel: Marker frequency in the original unselected population.
- FreqBottom: Marker frequency in the bottom sub-population in selective genotyping, if applicable.

Table 5.14 Results of selective genotyping (RSG, incomplete)

TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	LOD	FreqDiff	FreqTop	FreqUnsel	FreqBottom
1	Trait1	1	MK-1-1	1	0.0000	0.4513	0.0811	0.7864	0.7500	0.7052
1	Trait1	2	MK-1-2	1	10.0000	0.5735	0.0996	0.7935	0.7500	0.6939
1	Trait1	3	MK-1-3	1	20.0000	0.8210	0.1228	0.8050	0.7500	0.6822
1	Trait1	4	MK-1-4	1	30.0000	1.2206	0.1525	0.8209	0.7500	0.6684
1	Trait1	5	MK-1-5	1	40.0000	1.1850	0.1500	0.8212	0.7500	0.6712
1	Trait1	6	MK-1-6	1	50.0000	0.9072	0.1269	0.8150	0.7500	0.6881
1	Trait1	7	MK-1-7	1	60.0000	0.6288	0.1010	0.8016	0.7500	0.7006
1	Trait1	8	MK-1-8	1	70.0000	0.4392	0.0819	0.7935	0.7500	0.7116
1	Trait1	9	MK-1-9	1	80.0000	0.3514	0.0713	0.7859	0.7500	0.7146
1	Trait1	10	MK-1-10	1	90.0000	0.2887	0.0589	0.7804	0.7500	0.7215
1	Trait1	11	MK-1-11	1	100.0000	0.2129	0.0475	0.7718	0.7500	0.7242
1	Trait1	12	MK-2-1	2	0.0000	0.4196	0.0738	0.7860	0.7500	0.7122
1	Trait1	13	MK-2-2	2	10.0000	0.5426	0.0913	0.7947	0.7500	0.7035
1	Trait1	14	MK-2-3	2	20.0000	0.7135	0.1106	0.8058	0.7500	0.6951
1	Trait1	15	MK-2-4	2	30.0000	0.9930	0.1339	0.8164	0.7500	0.6825
1	Trait1	16	MK-2-5	2	40.0000	1.4909	0.1697	0.8319	0.7500	0.6621
1	Trait1	17	MK-2-6	2	50.0000	2.0853	0.2024	0.8497	0.7500	0.6474
1	Trait1	18	MK-2-7	2	60.0000	1.8576	0.1881	0.8440	0.7500	0.6559
1	Trait1	19	MK-2-8	2	70.0000	1.2685	0.1519	0.8276	0.7500	0.6757
1	Trait1	20	MK-2-9	2	80.0000	0.9251	0.1259	0.8165	0.7500	0.6906

……

5. LOD file: Average LOD score tesing only the epistatic varaiation during the two-dimensional scanning (Table 5.15)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Chromosome: Chromosome ID at the first scanning position.
- Position: Scanning position in cM of the first QTL.
- Others: LOD score between the two scanning positions.

Table 5.15 LOD score during the two-dimensional scanning (LOD, incomplete)

TraitID	TraitName	Chromosome	Position							
1	Trait1	1	0.0000							
1	Trait1	1	5.0000	0.0000						
1	Trait1	1	10.0000	0.0000	0.0000					
1	Trait1	1	15.0000	0.0000	0.0000	0.0000				
1	Trait1	1	20.0000	0.0000	0.0000	0.0000	0.0000			
1	Trait1	1	25.0000	0.1876	0.1902	0.2015	0.0000	0.0000		
1	Trait1	1	30.0000	0.1566	0.1583	0.1681	0.0000	0.0000	0.0000	
1	Trait1	1	35.0000	0.1524	0.1595	0.1744	0.1735	0.1801	0.0000	0.0000
1	Trait1	1	40.0000	0.1585	0.1726	0.1895	0.1828	0.1925	0.0000	0.0000	0.0000

……

6. IAA, IAD, IDA, and IDD files: average additive by additive effect, additive by dominance effect, dominance by additive effect, and dominance by dominance effect from the two-dimensional scanning of all populations. Format of these files is

the similar to that shown in Table 5.15.

5.4.3 Results files from significant QTL

1. QSM file: All significant markers from single marker analysis for all simulations (Table 5.16)

- Simulation: Simulation ID represented by an integer number.
- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- MarkerID: Marker ID represented by an integer number.
- MarkerName: Marker name, same as given in the BIP input file.
- Chromosome: Chromosome ID represented by an integer number.
- Position: Marker position in cM on the chromosome.
- LOD: LOD score calculated from single marker analysis.
- PVE(%): Phenotypic variation expelained by the marker.
- EstA: Estimated additive effect of the marker.
- EstD: Estimated dominance effect of the marker.

Table 5.16 Significant markers from single marker analysis (QSM, incomplete)

Simulation	TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	LOD	PVE(%)	EstA
1	1	Trait1	16	MK-2-5	2	40.0000	2.8634	6.3806	1.0497
1	1	Trait1	17	MK-2-6	2	50.0000	5.0243	10.9248	1.3767
1	1	Trait1	18	MK-2-7	2	60.0000	5.0373	10.9512	1.3806
1	1	Trait1	23	MK-3-1	3	0.0000	2.5395	5.6797	0.9910
1	1	Trait1	24	MK-3-2	3	10.0000	4.0823	8.9717	1.2461
1	1	Trait1	25	MK-3-3	3	20.0000	7.1246	15.1299	1.6165
1	1	Trait1	26	MK-3-4	3	30.0000	4.1668	9.1484	1.2573
1	1	Trait1	27	MK-3-5	3	40.0000	3.8538	8.4914	1.2137
1	1	Trait1	28	MK-3-6	3	50.0000	3.0902	6.8680	1.0916
1	1	Trait1	29	MK-3-7	3	60.0000	2.8286	6.3057	1.0486
1	2	Trait2	4	MK-1-4	1	30.0000	3.3647	7.4546	1.2820
1	2	Trait2	5	MK-1-5	1	40.0000	4.7318	10.3225	1.5045
1	2	Trait2	6	MK-1-6	1	50.0000	4.2915	9.4089	1.4402
1	2	Trait2	7	MK-1-7	1	60.0000	3.0050	6.6850	1.2094
1	2	Trait2	8	MK-1-8	1	70.0000	4.4473	9.7333	1.4551
1	2	Trait2	9	MK-1-9	1	80.0000	4.3131	9.4538	1.4366
1	2	Trait2	10	MK-1-10	1	90.0000	3.0662	6.8165	1.2296
1	2	Trait2	11	MK-1-11	1	100.0000	2.5678	5.7410	1.1250
1	2	Trait2	17	MK-2-6	2	50.0000	3.2140	7.1329	1.2447

……

2. QIM and QAD files: Significant QTL from simple interval mapping and ICIM-ADD for all simulations (significant additive and dominance QTL can be selected from this file; Table 5.17).

- Simulation: Simulation ID represented by an integer number.
- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Chromosome: Chromosome ID represented by an integer number.
- Position: The scanning position in cM on the chromosome.
- LeftMarker: Name of the left-side marker of the identified QTL.
- RightMarker: Name of the right-side marker of the identified QTL.
- LOD: LOD score.
- PVE(%): Phenotypic variation expelained by QTL at the current scanning position.

- EstA: Estimated additive effect of QTL at the current scanning position.
- EstD: Estimated dominance effect of QTL at the current scanning position.

Table 5.17 Significant QTL from SIM or ICIM-ADD (QIM or QAD, incomplete)

Simulation	TraitID	TraitName	Chromosome	Position	LeftMarker	RightMarker	LOD	PVE(%)	EstA
1	1	Trait1	2	55.0000	MK-2-6	MK-2-7	7.2522	14.6646	1.5943
1	1	Trait1	3	22.0000	MK-3-3	MK-3-4	8.7309	17.7320	1.7497
1	2	Trait2	1	43.0000	MK-1-5	MK-1-6	6.2758	13.0548	1.6916
1	2	Trait2	2	49.0000	MK-2-5	MK-2-6	4.6617	9.4210	1.4298
1	3	Trait3	1	25.0000	MK-1-3	MK-1-4	13.6160	22.2030	2.6498
1	3	Trait3	2	53.0000	MK-2-6	MK-2-7	11.9801	19.6627	2.4841
1	3	Trait3	3	24.0000	MK-3-3	MK-3-4	4.1743	6.5493	1.4290
1	3	Trait3	5	2.0000	MK-5-1	MK-5-2	2.9166	4.4564	-1.1789
1	4	Trait4	1	23.0000	MK-1-3	MK-1-4	2.8966	5.6544	0.9376
1	4	Trait4	2	45.0000	MK-2-5	MK-2-6	5.4146	11.3101	1.3202
1	4	Trait4	3	20.0000	MK-3-3	MK-3-4	6.3762	11.9280	1.3560
2	1	Trait1	1	8.0000	MK-1-1	MK-1-2	2.7466	5.4680	0.9935
2	1	Trait1	2	52.0000	MK-2-6	MK-2-7	3.5641	7.1797	1.1414
2	1	Trait1	3	21.0000	MK-3-3	MK-3-4	4.1292	8.2508	1.2196
2	2	Trait2	1	68.0000	MK-1-7	MK-1-8	15.0738	27.7748	2.5079
2	2	Trait2	2	53.0000	MK-2-6	MK-2-7	6.2608	11.0086	1.5828
2	3	Trait3	1	33.0000	MK-1-4	MK-1-5	4.5566	8.5767	1.4157
2	3	Trait3	2	54.0000	MK-2-6	MK-2-7	8.9167	17.8550	2.0447
2	3	Trait3	3	10.0000	MK-3-2	MK-3-3	4.8093	8.0958	1.3736
2	4	Trait4	2	54.0000	MK-2-6	MK-2-7	6.8088	13.5564	1.5325
2	4	Trait4	3	15.0000	MK-3-2	MK-3-3	9.1362	17.8849	1.7541

……

3. QEP file: All significant peaks from two-dimensional scanning for all simulations (Significant digenic interacting QTL from ICIM can be selected from this file; Part 1 is in Table 5.18, and Part 2 is similar to Table 5.12).

- Simulation: Simulation ID represented by an integer number.
- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Chromosome1: Chromosome ID at the first scanning position.
- Position1: Scanning position in cM of the first QTL.
- Chromosome2: Chromosome ID at the second scanning position.
- Position2: Scanning position in cM of the second QTL.
- LOD: LOD score caused by epistasis effects.
- PVE: Phenotypic variation expelained by epsitatic QTL effects.

- EstA1: Estimated additive effect of the first QTL.
- EstA2: Estimated additive effect of the second QTL.
- EstD1: Estimated dominance effect of the first QTL.
- EstD2: Estimated dominance effect of the second QTL.
- EstAA: Estimated additive by additive effect of QTL at the two scanning positions.
- EstAD: Estimated additive by dominance effect of QTL at the two scanning positions.
- EstDA: Estimated dominance by additive effect of QTL at the two scanning positions.
- EstDD: Estimated dominance by dominance effect of QTL at the two scanning positions.

Table 5.18 Significant interactions from ICIM-EPI Part 1 (QEP, incomplete)

Simulation	TraitID	TraitName	Chromosome1	Position1	LeftMarker1	RightMarker1	Chromosome2	Position2	LeftMarker2	RightMarker2	LOD	PVE	EstA1	EstA2	EstAA
6	3	Trait3	1	35.0000	MK-1-4	MK-1-5	2	50.0000	MK-2-6	MK-2-7	5.5947	7.4197	1.1021	-1.2872	2.9510
8	2	Trait2	4	25.0000	MK-4-3	MK-4-4	5	70.0000	MK-5-8	MK-5-9	5.1760	8.7669	1.1506	1.9189	-2.9830
13	4	Trait4	1	30.0000	MK-1-4	MK-1-5	2	65.0000	MK-2-7	MK-2-8	6.7439	10.8931	-0.7140	-1.5146	2.8032
22	4	Trait4	1	30.0000	MK-1-4	MK-1-5	2	55.0000	MK-2-6	MK-2-7	9.2845	14.5089	-1.0966	-0.6374	3.2816
25	4	Trait4	1	60.0000	MK-1-7	MK-1-8	2	50.0000	MK-2-6	MK-2-7	6.0267	9.8744	-1.0721	-0.5364	2.4410
26	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	55.0000	MK-2-6	MK-2-7	5.4361	8.8608	-0.2680	0.4480	2.4417
29	3	Trait3	1	40.0000	MK-1-5	MK-1-6	2	55.0000	MK-2-6	MK-2-7	6.8071	9.3712	0.1425	0.7591	3.2938
38	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	50.0000	MK-2-6	MK-2-7	5.6212	8.7658	-1.2282	0.1695	2.4435
39	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	50.0000	MK-2-6	MK-2-7	5.7642	8.5922	-0.8561	-0.2971	2.5016
40	3	Trait3	1	40.0000	MK-1-5	MK-1-6	2	55.0000	MK-2-6	MK-2-7	5.9604	7.6155	0.8614	0.7684	2.9985
43	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	45.0000	MK-2-5	MK-2-6	6.7186	13.1090	-0.0952	-0.6246	2.7392
44	4	Trait4	1	40.0000	MK-1-5	MK-1-6	2	50.0000	MK-2-6	MK-2-7	5.3973	7.5816	-0.3398	-0.0705	2.1451
46	3	Trait3	1	35.0000	MK-1-4	MK-1-5	2	55.0000	MK-2-6	MK-2-7	5.7643	8.1104	0.3730	0.8534	3.3248
47	4	Trait4	1	45.0000	MK-1-5	MK-1-6	2	60.0000	MK-2-7	MK-2-8	6.8792	12.2777	-0.3610	-0.6141	2.7542
50	4	Trait4	1	45.0000	MK-1-5	MK-1-6	2	60.0000	MK-2-7	MK-2-8	5.3639	9.9549	-0.1765	-0.8417	2.4368
53	3	Trait3	1	25.0000	MK-1-3	MK-1-4	2	50.0000	MK-2-6	MK-2-7	5.0584	7.4668	-0.8302	0.3583	2.6889
54	4	Trait4	1	30.0000	MK-1-4	MK-1-5	2	50.0000	MK-2-6	MK-2-7	6.9304	12.0933	-0.6420	-0.3710	2.5831
55	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	55.0000	MK-2-6	MK-2-7	9.1397	15.9663	-0.2940	-1.0266	2.9207
56	3	Trait3	1	40.0000	MK-1-5	MK-1-6	2	65.0000	MK-2-7	MK-2-8	7.0507	10.9155	-0.3438	0.4259	3.4779
56	4	Trait4	1	40.0000	MK-1-5	MK-1-6	2	60.0000	MK-2-7	MK-2-8	5.2901	9.0990	-0.6221	-0.2193	2.4090
59	4	Trait4	1	30.0000	MK-1-4	MK-1-5	2	45.0000	MK-2-5	MK-2-6	7.1690	11.5324	-0.5788	-0.2144	2.8962
60	4	Trait4	1	25.0000	MK-1-3	MK-1-4	2	60.0000	MK-2-7	MK-2-8	6.3514	12.5232	-0.4925	-0.7483	2.6742
61	4	Trait4	1	40.0000	MK-1-5	MK-1-6	2	55.0000	MK-2-6	MK-2-7	5.8558	10.3622	-1.2018	-0.9761	2.5744
64	1	Trait1	2	65.0000	MK-2-7	MK-2-8	3	75.0000	MK-3-8	MK-3-9	5.8503	10.1625	2.8842	1.4996	-2.8203
64	4	Trait4	1	45.0000	MK-1-5	MK-1-6	2	55.0000	MK-2-6	MK-2-7	6.6386	13.5244	-0.8896	-0.1056	2.8170
67	3	Trait3	1	50.0000	MK-1-6	MK-1-7	2	50.0000	MK-2-6	MK-2-7	5.8069	7.2245	-1.2605	0.5935	2.9243
70	4	Trait4	1	40.0000	MK-1-5	MK-1-6	2	60.0000	MK-2-7	MK-2-8	5.9848	8.8660	-0.2774	-1.1970	2.3330
77	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	50.0000	MK-2-6	MK-2-7	5.1557	9.5186	-0.1635	-0.4634	2.2773
79	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	55.0000	MK-2-6	MK-2-7	7.1018	13.3067	-0.1840	-1.4008	2.8770
79	4	Trait4	1	45.0000	MK-1-5	MK-1-6	2	70.0000	MK-2-8	MK-2-9	6.3433	10.9114	-1.0912	-1.4203	2.6229
80	3	Trait3	1	30.0000	MK-1-4	MK-1-5	2	50.0000	MK-2-6	MK-2-7	5.1220	7.3803	-1.5270	0.5762	3.0231
80	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	70.0000	MK-2-8	MK-2-9	5.8639	10.2212	-0.2634	-0.9328	2.8315
86	4	Trait4	1	40.0000	MK-1-5	MK-1-6	2	50.0000	MK-2-6	MK-2-7	6.4733	10.3472	-0.1824	-0.4710	2.7620
87	3	Trait3	1	45.0000	MK-1-5	MK-1-6	2	60.0000	MK-2-7	MK-2-8	5.6631	8.8079	-0.3096	0.3717	3.0144
91	4	Trait4	1	45.0000	MK-1-5	MK-1-6	2	60.0000	MK-2-7	MK-2-8	5.2923	9.4185	-0.3292	-0.3584	2.5076
92	4	Trait4	1	40.0000	MK-1-5	MK-1-6	2	50.0000	MK-2-6	MK-2-7	5.5747	7.3623	-0.4931	0.0139	1.9986
94	4	Trait4	1	30.0000	MK-1-4	MK-1-5	2	50.0000	MK-2-6	MK-2-7	5.9194	10.4625	-0.9389	0.1357	2.6342
95	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	50.0000	MK-2-6	MK-2-7	5.3207	10.0699	-0.7626	-0.8521	2.3630
97	4	Trait4	1	35.0000	MK-1-4	MK-1-5	2	45.0000	MK-2-5	MK-2-6	6.5977	10.7780	-0.5711	-0.1059	2.7023

……

4. QSG file: All significant peaks from selective genotyping for all simulations (Table 5.19)

- Simulation: Simulation ID represented by an integer number.
- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- MarkerID: Marker ID represented by an integer number.
- MarkerName: Marker name, same as given in the BIP input file.
- Chromosome: Chromosome ID represented by an integer number.
- Position: Marker position in cM on the chromosome.
- LOD: LOD score calculated from selective genotyping.
- FreqDiff: Difference in marker frequency in the two sub-populations in selective genotyping.

Table 5.19 Significant interactions from selective genotyping (QSG, incomplete)

Simulation	TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	LOD	FreqDiff
1	3	Trait3	4	MK-1-4	1	30.0000	6.6116	0.3125
2	2	Trait2	4	MK-1-4	1	30.0000	5.4536	0.3250
2	2	Trait2	7	MK-1-7	1	60.0000	5.7299	0.3250
2	2	Trait2	8	MK-1-8	1	70.0000	6.1298	0.3500
3	1	Trait1	25	MK-3-3	3	20.0000	6.4639	0.3250
4	1	Trait1	25	MK-3-3	3	20.0000	6.4106	0.3375
4	1	Trait1	26	MK-3-4	3	30.0000	5.7165	0.3125
4	2	Trait2	8	MK-1-8	1	70.0000	5.0161	0.3250
4	3	Trait3	4	MK-1-4	1	30.0000	5.7299	0.3250
4	3	Trait3	17	MK-2-6	2	50.0000	5.0679	0.3000
5	3	Trait3	4	MK-1-4	1	30.0000	5.2185	0.3250
5	3	Trait3	17	MK-2-6	2	50.0000	5.4536	0.3250
6	2	Trait2	4	MK-1-4	1	30.0000	5.4536	0.3250
6	3	Trait3	5	MK-1-5	1	40.0000	5.0500	0.2875
7	3	Trait3	17	MK-2-6	2	50.0000	5.3952	0.3125
7	3	Trait3	18	MK-2-7	2	60.0000	5.7299	0.3250
9	3	Trait3	17	MK-2-6	2	50.0000	6.7778	0.3625
10	1	Trait1	25	MK-3-3	3	20.0000	5.1269	0.3125
10	3	Trait3	5	MK-1-5	1	40.0000	5.3952	0.3125

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5.4.4 Results files from power analysis

The following files are only for power analysis.

1. PSM: Power analysis of single marker analysis (Table 5.20)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- MarkerID: Marker ID represented by an integer number.
- MarkerName: Marker name, same as given in the BIP input file.
- Chromosome: Chromosome ID represented by an integer number.
- Position: Marker position in cM on the chromosome.
- Power: Detection power of QTL in simulation.
- LOD: LOD score calculated from single marker analysis. NaN (not a number) in Fortran means the value cannot be determined.
- SE_LOD: Standard error of LOD score calculated from single marker analysis.
- EstA: Estimated additive effect of the marker.
- SE_EstA: Standard error of estimated additive effect of the marker.

Table 5.20 information in PSM file (incomplete)

TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	Power	LOD	SE_LOD	EstA	SE_EstA
1	Trait1	1	MK-1-1	1	0.0000	2	2.5314	0.0036	1.0224	0.0460
1	Trait1	2	MK-1-2	1	10.0000	8	2.9492	0.1573	1.0879	0.0415
1	Trait1	3	MK-1-3	1	20.0000	17	3.4526	0.6935	1.1773	0.1118
1	Trait1	4	MK-1-4	1	30.0000	34	3.7862	1.1181	1.2110	0.1685
1	Trait1	5	MK-1-5	1	40.0000	36	3.5644	0.9005	1.1881	0.1621
1	Trait1	6	MK-1-6	1	50.0000	20	3.2227	0.6618	1.1390	0.1208
1	Trait1	7	MK-1-7	1	60.0000	8	2.9967	0.4700	1.0823	0.0676
1	Trait1	8	MK-1-8	1	70.0000	4	2.8500	0.2174	1.0883	0.0303
1	Trait1	9	MK-1-9	1	80.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	10	MK-1-10	1	90.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	11	MK-1-11	1	100.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	12	MK-2-1	2	0.0000	3	2.7198	0.1391	1.0394	0.0500
1	Trait1	13	MK-2-2	2	10.0000	7	3.0708	0.8225	1.1048	0.1158
1	Trait1	14	MK-2-3	2	20.0000	12	3.3874	0.8477	1.1543	0.1682
1	Trait1	15	MK-2-4	2	30.0000	27	3.5946	0.7058	1.1840	0.1243
1	Trait1	16	MK-2-5	2	40.0000	56	3.8568	1.1339	1.2202	0.1721
1	Trait1	17	MK-2-6	2	50.0000	81	4.6018	1.5047	1.3200	0.2192
1	Trait1	18	MK-2-7	2	60.0000	70	4.3753	1.3224	1.2937	0.1962
1	Trait1	19	MK-2-8	2	70.0000	42	3.6423	0.9593	1.1970	0.1433
1	Trait1	20	MK-2-9	2	80.0000	23	3.3989	0.6061	1.1581	0.1106

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2. PIM and PAD: Power analysis of simple interval mapping and ICIM-ADD. The first part contains information for each QTL (Table 5.21), and the second part contains information for each marker interval (Table 5.22).

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Chromosome: Chromosome ID represented by an integer number.
- Power: Detection power of QTL in simulation.
- EstPOS: The estimated position in cM of QTL on the chromosome.
- SE_POS: Standard error of the estimated position in cM of QTL on the chromosome.
- LOD: LOD score calculated from interval mapping.
- SE_LOD: Standard error of LOD score calculated from interval mapping.
- EstA: Estimated additive effect of QTL at the current scanning position.
- SE_EstA: Standard error of estimated additive effect of QTL at the current scanning position.

Table 5.21 information in PIM file (Part 1, incomplete)

TraitID	TraitName	Chromosome	QTL	Power	EstPOS	SE_POS	LOD	SE_LOD	EstA	SE_EstA
1	Trait1	1	QTL001	31	34.6774	3.5231	4.2026	1.2115	1.3175	0.1947
1	Trait1	2	QTL002	65	52.8462	2.7244	4.8231	1.6400	1.3907	0.2462
1	Trait1	3	QTL003	74	21.5811	2.5416	6.4749	2.2531	1.5800	0.2677
1	Trait1	FalseQTL	FalseQTL	85						
2	Trait2	1	QTL001	27	35.2222	3.0103	6.8246	1.9516	1.8177	0.3019
2	Trait2	1	QTL002	66	68.9394	2.6105	7.4761	2.4161	1.9109	0.3276
2	Trait2	2	QTL003	53	53.2642	2.6073	5.1225	1.5493	1.6238	0.2537
2	Trait2	FalseQTL	FalseQTL	94						
3	Trait3	1	QTL001	86	35.1977	2.6756	6.9564	2.1707	2.0710	0.3406
3	Trait3	2	QTL002	90	53.2111	2.4922	8.6311	2.3532	2.2833	0.3247
3	Trait3	3	QTL003	44	21.4545	2.5713	4.7207	1.3821	1.7259	0.2756
3	Trait3	FalseQTL	FalseQTL	92						
4	Trait4	1	QTL001	38	34.0789	3.4212	3.9679	1.2534	1.2069	0.1822
4	Trait4	2	QTL002	35	52.3429	2.8278	4.3145	1.4785	1.2372	0.2142
4	Trait4	3	QTL003	79	21.3797	2.5373	6.8474	2.1901	1.5390	0.2574
4	Trait4	FalseQTL	FalseQTL	78						

Table 5.22 information in PIM file (Part 2, incomplete)

TraitID	TraitName	MarkerID	MarkerName	Power	EstPOS	SE_POS	LOD	SE_LOD	EstA	SE_EstA
1	Trait1	1	MK-1-1	0	NaN	0.0000	NaN	0.0000	NaN	0.0000
1	Trait1	2	MK-1-2	2	16.5000	1.5000	3.9597	0.4280	1.3379	0.0229
1	Trait1	3	MK-1-3	4	25.7500	0.8292	3.5443	0.9230	1.1792	0.1438
1	Trait1	4	MK-1-4	27	33.8889	3.0712	4.2629	1.2652	1.3238	0.2055
1	Trait1	5	MK-1-5	10	42.8000	3.3106	3.7114	0.5374	1.2427	0.0744
1	Trait1	6	MK-1-6	1	50.0000	0.0000	4.7466	0.0000	1.3648	0.0000
1	Trait1	7	MK-1-7	1	60.0000	0.0000	4.1616	0.0000	1.2113	0.0000
1	Trait1	8	MK-1-8	1	70.0000	0.0000	2.6932	0.0000	1.1002	0.0000
1	Trait1	9	MK-1-9	0	NaN	0.0000	NaN	0.0000	NaN	0.0000
1	Trait1	10	MK-1-10	0	NaN	0.0000	NaN	0.0000	NaN	0.0000
1	Trait1	11	MK-1-11							
1	Trait1	12	MK-2-1	2	2.0000	2.0000	2.7005	0.1717	1.0247	0.0855
1	Trait1	13	MK-2-2	3	14.3333	2.0548	3.6563	1.1681	1.1977	0.1692
1	Trait1	14	MK-2-3	1	28.0000	0.0000	2.9662	0.0000	1.0844	0.0000
1	Trait1	15	MK-2-4	6	34.1667	3.4359	3.5744	0.8339	1.1793	0.1032
1	Trait1	16	MK-2-5	14	45.5000	2.6118	5.2994	1.5120	1.4681	0.2234
1	Trait1	17	MK-2-6	63	53.3333	2.6963	4.8010	1.6710	1.3867	0.2509
1	Trait1	18	MK-2-7	9	61.3333	2.2608	5.1987	1.4000	1.4169	0.2180
1	Trait1	19	MK-2-8	1	70.0000	0.0000	3.2304	0.0000	1.1670	0.0000
1	Trait1	20	MK-2-9	2	82.0000	2.0000	3.6838	0.8734	1.1994	0.2594

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3. PEP: Power analysis of two dimensional ICIM (Part 1 in Table 5.23, and Part 2 in Table 5.24).

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- QTLCh1: Chromosome ID at the first QTL.
- QTLID1: To represent QTL ID at the first QTL.
- QTLCh2: Chromosome ID at the second QTL.
- QTLID2: To represent QTL ID at the second QTL.
- Power: Detection power of QTL in simulation.
- EstPOS1: Estimated position in cM of the first QTL.
- SE_POS1: Standard error of estimated position in cM of the first QTL.
- EstPOS2: Estimated position in cM of the second QTL.
- SE_POS2: Standard error of estimated position in cM of the second QTL.

- LOD: LOD score caused by epistasis effects.
- SE_LOD: Standard error of LOD score caused by epistasis effects.
- EstA1: Estimated additive effect of the first QTL.

- SE_EstA1: Standard error of estimated additive effect of the first QTL.
- EstA2: Estimated additive effect of the second QTL.
- SE_EstA2: Standard error of estimated additive effect of the second QTL.
- EstAA: Estimated additive by additive effect of QTL at the two scanning positions.
- SE_EstAA: Standard error of estimated additive by additive effect of QTL at the two scanning positions.

Table 5.23 information in PEP file (Part 1, incomplete)

TraitID	TraitName	QTL1Ch	QTL1ID	QTL2Ch	QTL2ID	Power	EstPOS1	SE_POS1	EstPOS2	SE_POS2
1	Trait1	1	1	2	2	0	NaN	0.0000	NaN	0.0000
1	Trait1	1	1	3	3	0	NaN	0.0000	NaN	0.0000
1	Trait1	2	2	3	3	0	NaN	0.0000	NaN	0.0000
1	Trait1	FalseQTL	FalseQTL	FalseQTL	FalseQTL	1				
2	Trait2	1	1	1	2	0	NaN	0.0000	NaN	0.0000
2	Trait2	1	1	2	3	0	NaN	0.0000	NaN	0.0000
2	Trait2	1	2	2	3	0	NaN	0.0000	NaN	0.0000
2	Trait2	FalseQTL	FalseQTL	FalseQTL	FalseQTL	1				
3	Trait3	1	1	2	2	5	36.0000	3.7417	53.0000	2.449
3	Trait3	1	1	3	3	0	NaN	0.0000	NaN	0.0000
3	Trait3	2	2	3	3	0	NaN	0.0000	NaN	0.0000
3	Trait3	FalseQTL	FalseQTL	FalseQTL	FalseQTL	4				
4	Trait4	1	1	2	2	14	35.3571	3.5174	51.7857	2.3958
4	Trait4	1	1	3	3	0	NaN	0.0000	NaN	0.0000
4	Trait4	2	2	3	3	0	NaN	0.0000	NaN	0.0000
4	Trait4	FalseQTL	FalseQTL	FalseQTL	FalseQTL	14				

Table 5.24 information in PEP file (Part 2, incomplete)

LOD	SE_LOD	EstA1	SE_EstA1	EstA2	SE_EstA2	EstAA	SE_EstAA
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
5.8497	0.5533	0.1904	0.9238	0.3340	0.8156	3.1182	0.1580
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
6.3553	1.2941	-0.6179	0.3807	-0.4142	0.5081	2.5574	0.3202
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
0.0000	NaN	0.0000	NaN	0.0000	NaN	0.0000	NaN
……							

4. PSG: Power analysis of selective genotyping (Table 5.25)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- MarkerID: Marker ID represented by an integer number.
- MarkerName: Marker name, same as given in the BIP input file.
- Chromosome: Chromosome ID represented by an integer number.
- Position: Marker position in cM on the chromosome.
- Power: Detection power of QTL in simulation.
- LOD: LOD score calculated from selective genotyping.
- SE_LOD: Standard error of LOD score calculated from selective genotyping.
- FreqDiff: Difference in marker frequency in the two sub-populations in selective genotyping.
- SE_FreqDiff: Standard error of difference in marker frequency in the two

sub-populations in selective genotyping.

Table 5.25 information in PSG file (incomplete)

TraitID	TraitName	MarkerID	MarkerName	Chromosome	Position	Power	LOD	SE_LOD	FreqDiff	SE_FreqDiff
1	Trait1	1	MK-1-1	1	0.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	2	MK-1-2	1	10.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	3	MK-1-3	1	20.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	4	MK-1-4	1	30.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	5	MK-1-5	1	40.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	6	MK-1-6	1	50.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	7	MK-1-7	1	60.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	8	MK-1-8	1	70.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	9	MK-1-9	1	80.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	10	MK-1-10	1	90.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	11	MK-1-11	1	100.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	12	MK-2-1	2	0.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	13	MK-2-2	2	10.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	14	MK-2-3	2	20.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	15	MK-2-4	2	30.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	16	MK-2-5	2	40.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	17	MK-2-6	2	50.0000	1	5.0161	0.0008	0.3250	0.0000
1	Trait1	18	MK-2-7	2	60.0000	1	6.8241	0.0007	0.3375	0.0000
1	Trait1	19	MK-2-8	2	70.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	20	MK-2-9	2	80.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	2	MK-2-10	2	90.0000	0	NaN	0.0000	NaN	0.0000
1	Trait1	2	MK-2-11	2	100.0000	0	NaN	0.0000	NaN	0.0000

……

5. BIP: If “Outputing mapping population” in Parameter windows (Figure 5.1) is checked, all simulated population will be output in the BIP format, which can then be uploaded as any other actual BIP mapping population.

5.5 Figures

Figures from BIP simulation are similar to those of BIP mapping. All figures from simulation are based on the average results across all runs.

5.5.1 Figures from ICIM additive mapping (ICIM-ADD)

Click tool bar 

or ICIM for additive mapping in the Figures menu to draw graphs from the ICIM-ADD method. A new window with the title “[Graph] ICIM for additive mapping - …” will be open (Figures 5.7 and 5.8). Tool bars are available to change the figure format. Purposes of these tool bars are explained in Figure 5.7. Graphs can be shown one by one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 5.8).

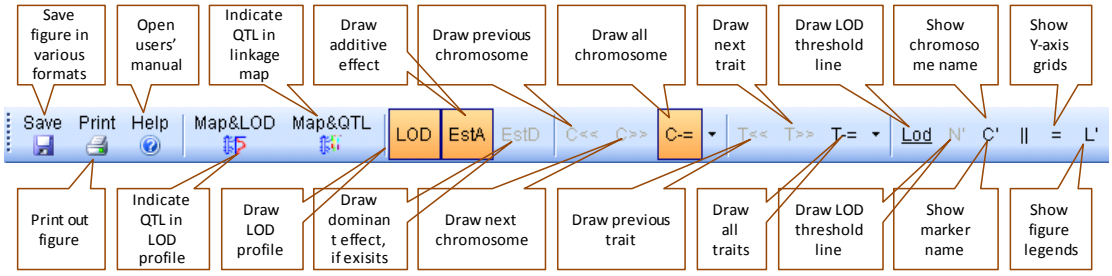

Figure 5.7 Tool bars in figures from ICIM additive mapping (ICIM-ADD)

The software also provides figures to combine LOD score with the linkage map (Figure 4.9A), and identified QTL with linkage map (Figure 4.9B). As the graphs for the LOD score / estimated genetic effects, the combined figures can also be shown one by one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 4.9).

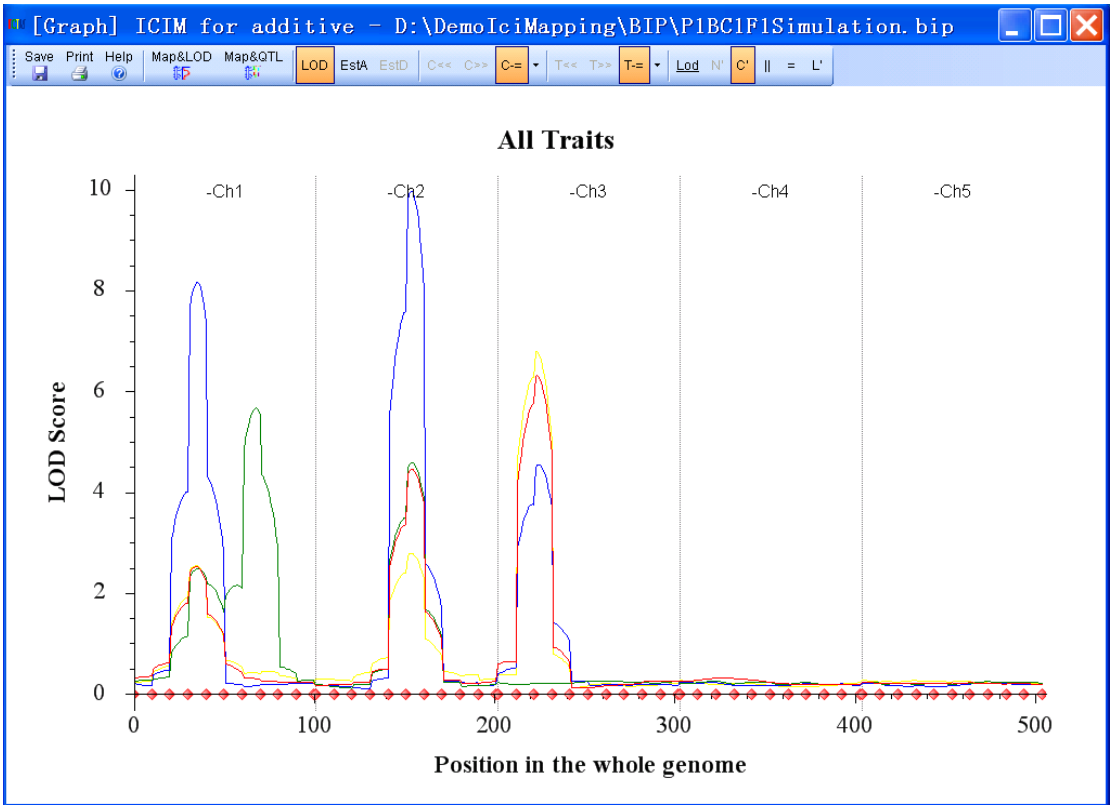

Figure 5.8 Figures from ICIM additive mapping (ICIM-ADD)

A. Combined figure of linkage map and LOD score

B. Combined figure of linkage map and identified QTL

Figure 5.9 Combined Figure from ICIM additive mapping (ICIM-ADD)

5.5.2 Figures from ICIM epistatic mapping (ICIM-EPI)

Click tool bar 

or ICIM for epistatic mapping in the Figures menu to draw graphs from the ICIM-EPI method. A new window with the title "[Graph] ICIM for epistatic - …" will be open (Figure 4.10). Tool bars are available to change the figure format. Graphs are shown for all chrosmomes.

5.5.3 Figures from simple interval mapping (SIM)

Click Simple interval mapping in the Figures menu to draw graphs from the SIM method. A new window with the title "[Graph] Simple interval mapping - …" will be open (Figure 4.11). Same tool bars as ICIM-ADD are available to change the figure format. Purposes of these tool bars are explained in Figure 4.7. Graphs can be shown one by one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 4.11).

5.5.4 Figures from single marker analysis (SMA)

Click Single marker analysis in the Figures menu to draw graphs from the SMA method. A new window with the title "[Graph] Single marker analysis - …" will be open to show the bar graph (Figure 4.12). Similar tool bars as ICIM-ADD are available to change the figure format. Graphs can be shown for markers on one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 4.12).

5.5.5 Figures from selective genotyping mapping (SGM)

Click Selective genotyping in the Figures menu to draw graphs from the SGM method. A new window with the title "[Graph] Selective genotyping - …" will be open to show the bar graph (Figure 4.13). Same tool bars as SMA are available to change the figure format. Graphs can be shown for markers on one chromosome, or for all chrosmomes, or for selected chromosomes (Figure 4.13).

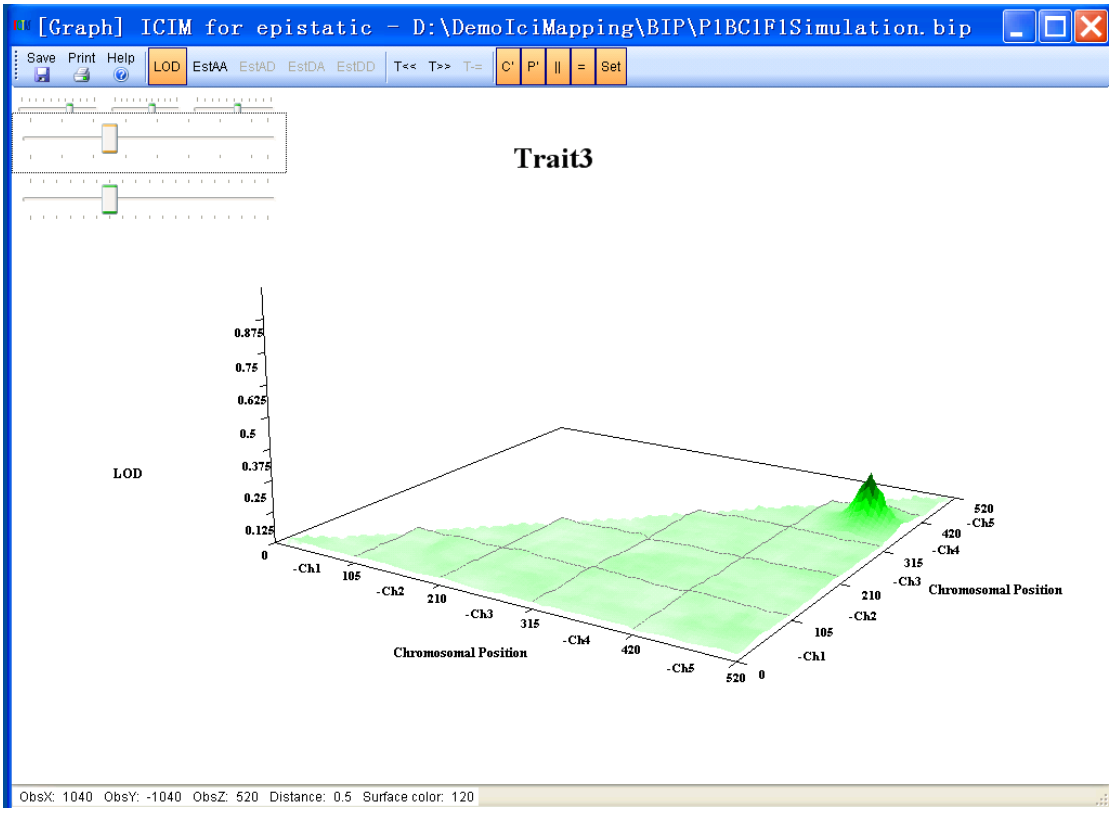

Figure 5.10 Figures from ICIM epistatic mapping (ICIM-EPI)

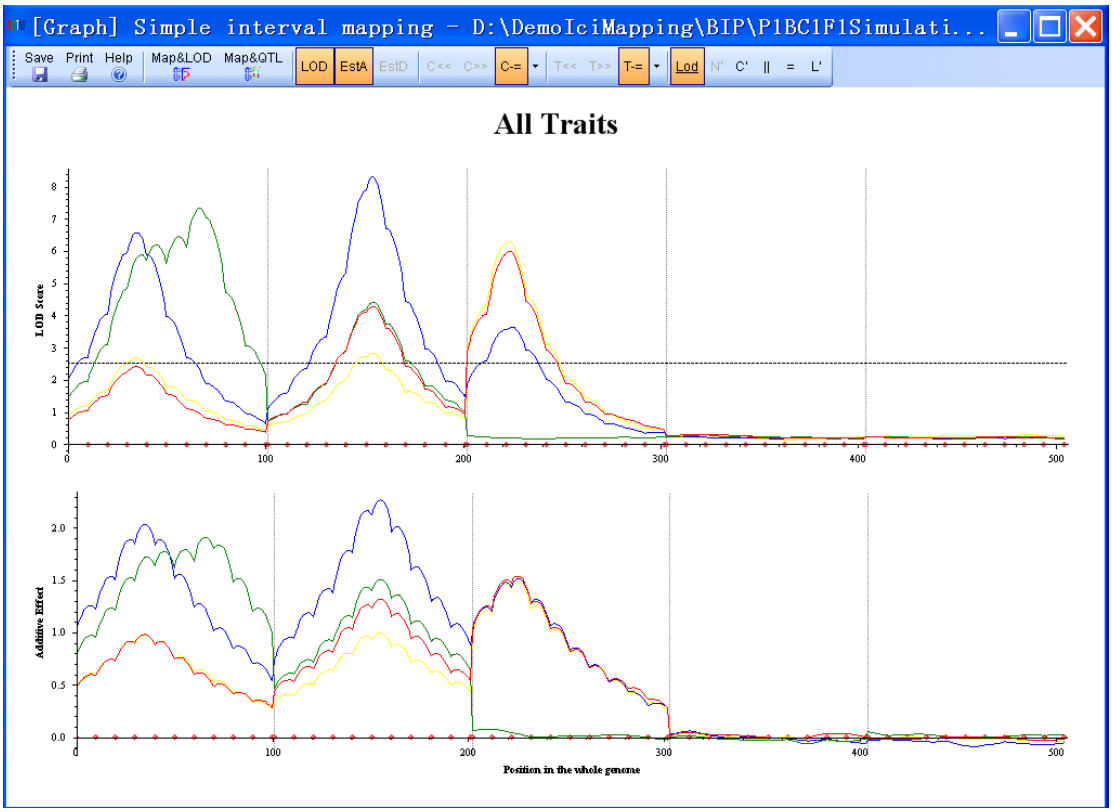

Figure 5.11 Figures from simple interval mapping (SIM)

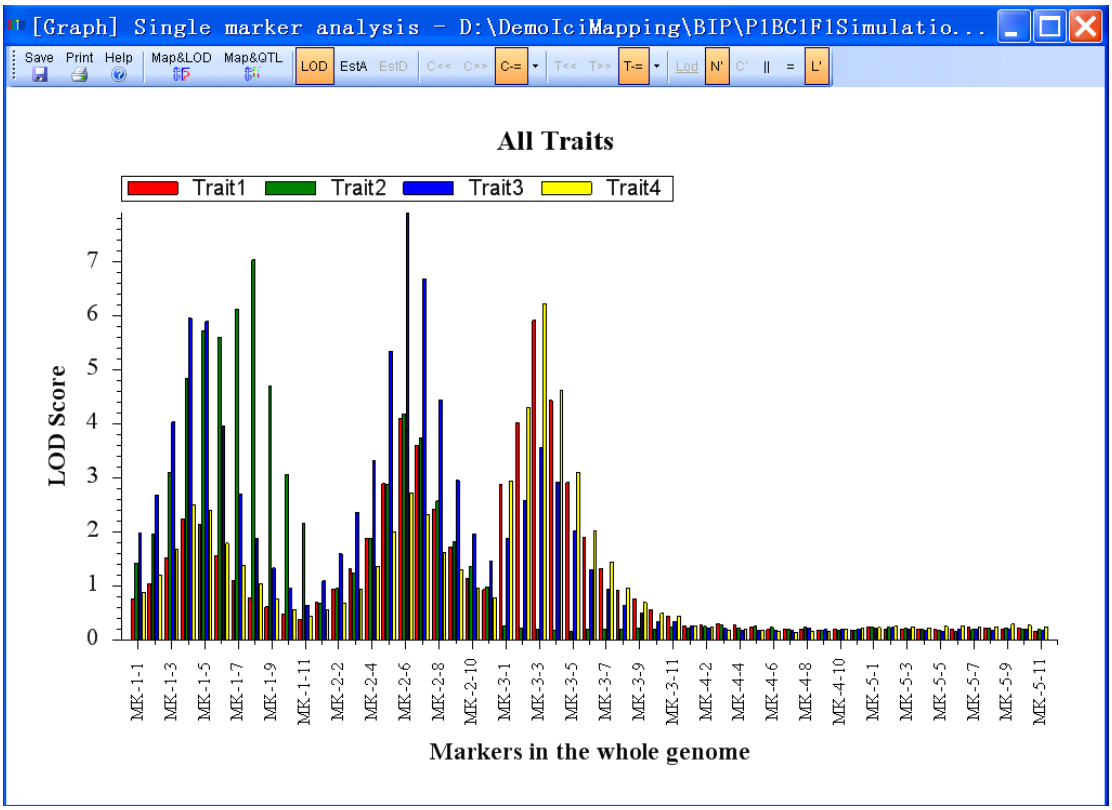

Figure 5.12 Figures from single marker analysis (SMA)

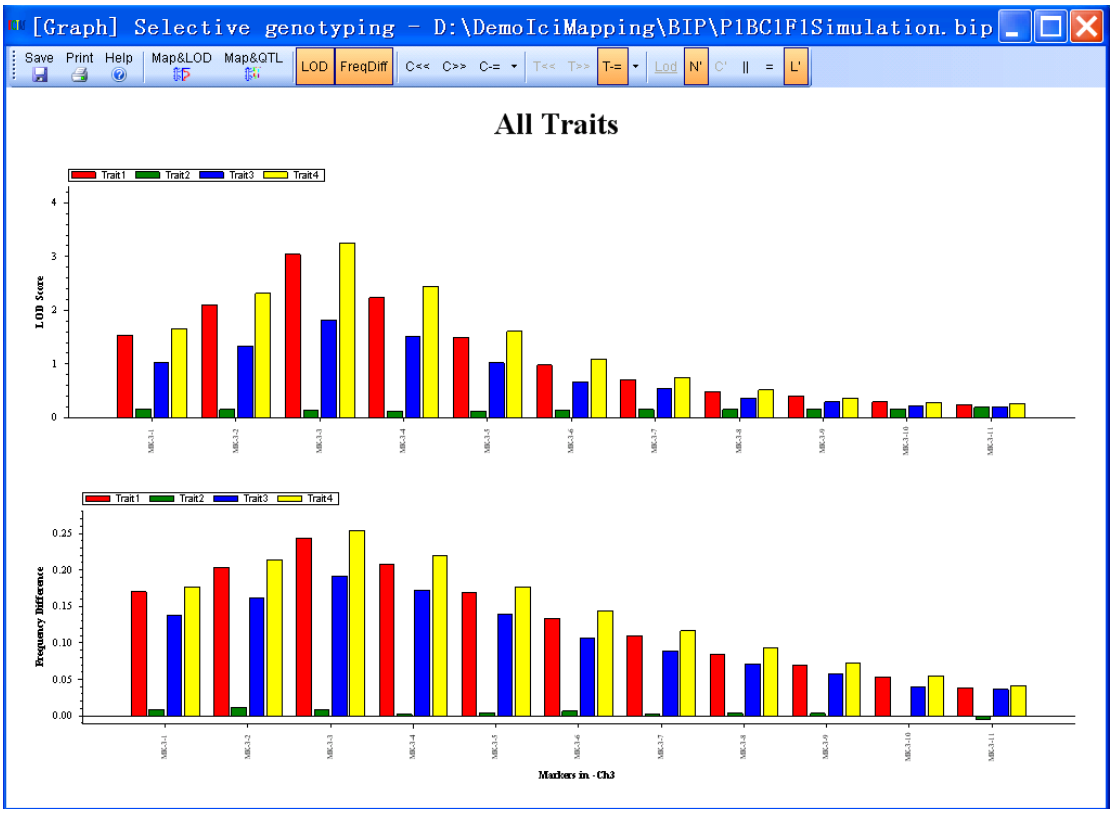

Figure 5.13 Figures from selective genotyping mapping (SGM)

Chapter 6. QTL Mapping with CSS Lines (CSL)

By using QTL IciMapping, one can also conduct QTL mapping for CSS lines, or conduct power analysis for a set of predefined QTL so as to compare the efficiency of different mappingmethods. We introduce QTL mapping with CSS Lines in this chapter. There are three mapping methods in the software: single marker analysis for CSS Lines, RSTEP-LRT-ADD and RSTEP-LRT-EPI.

One mapping population is defined in an input file with the extension name ‘csl’. Tables 6.1-6.3 give a working example with 66 lines. Three parts can be seen in Tables 6.1-6.3, respectively, i.e. the general information for the mapping population, marker types, and phenotypes. Lines starting with ‘!’ are remarks and will be ignored in the QTL IciMapping software.

6.1 Input file for QTL mapping with CSS Lines (*.csl)

6.1.1 General population information

Six parameters were used for the general information describing a mapping population (Table 6.1).

- Indicator: let IciMapping know if a mapping study or power simulation will be conducted. Indicator 1 has to be assigned for any QTL mapping studies (Table 6.1).
  - 1 for a mapping study.
  - 2 for a power simulation.
- Number of donor chromosome segments: Specify the number of donor chromosome segments in these CSS lines.
- Number of CSS Lines: number of CSS lines in the mapping population. It has been proved that the inclusion of the background will reduce the marker collinerarity and therefore improve the mapping power. For example, if 65 CSS lines and their background parent were used in QTL mapping, the number of 66 has to be specified.
- Number of traits: number of traits in the mapping population.
- Number of testing environments: number of testing environments to phenotype the trait in interest.
- Number of replications: number of replications in each testing environment.

Table 6.1 General population information in a QTL mapping input file.

```
!******************** Input file for QTL mapping with CSS lines **********************
!Lines starting with '!' are remarks and will be ignored when read.
1       !Purpose: 1 for trait mapping, 2 for mapping simulation;
82      !Number of donor chromosome segments (represented by markers)
66      !Number of CSS lines plus the recurrent (or background) parent
```

```
1        !Number of traits,            if Purpose=1
4        !Number of testing environments, if Purpose=1
2        !Number or replications,       if Purpose=1
```

6.1.2 Marker type information

Marker types for all individuals on the first marker were given first, and then followed by the second marker, and so on. In IciMapping (Table 6.2), 2 was used for the marker type of the donor parent, 0 for the background parent, and -1 for any missing marker. Missing markers will be assigned to the value 0 in QTL mapping. The number of marker types behind each marker name has to be equal to the number of CSS lines.

Table 6.2 Marker type information in a QTL mapping input file.

```
!***************** Marker type: 0 for background parent (aa), 2 for donor parent (AA), -1 for missing marker ************************
!Marker name followed by marker type for all lines (the kackground parent and CSS lines)
M1  0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M2  0 2 2 2 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M3  0 2 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M4  0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M5  0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0
M6  0 0 0 0 0 2 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M7  0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0
M8  0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0
M9  0 0 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M10 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M11 0 0 0 0 0 0 0 2 0 0 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M12 0 0 0 0 0 0 0 2 0 0 2 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M13 0 0 0 0 0 0 0 2 0 0 2 2 2 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M14 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M15 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M16 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M17 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0
M18 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0
M19 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M20 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M21 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M22 0 2 2 0 0 0 0 0 0 2 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M23 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M24 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M25 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M26 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M27 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M28 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M29 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M30 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M31 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M32 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 2 2 2 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M33 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0
M34 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M35 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M36 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 2 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M37 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M38 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M39 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M40 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M41 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0
M42 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M43 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0
M44 0 0 0 0 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 2 0
M45 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 2 0
M46 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M47 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M48 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 2
M49 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0
M50 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M51 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M52 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M53 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M54 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 2 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M55 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M56 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M57 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M58 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M59 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 2 0 0
M60 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2 0 2 2 0 0 0 0 0 0 0 0 0 0 0
M61 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0
M62 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 2 0 0 0 0 0 0 0 0 0
M63 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0
M64 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0
M65 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 2 0 0 0 0 0 0 0
M66 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0
M67 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0
M68 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0
M69 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 2 0 0 0 0 2 0 2 0 0 0 0 0 0 2 0 0 0 0 0 0 0
M70 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0
M71 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0
M72 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0
M73 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0
M74 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0
M75 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0
M76 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0
M77 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 2 0 2 2 2 0 0 0 0 0 0 2 0 0 0 2 2
M78 0 0 0 0 0 0 2 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 2 0 0 0 2 2
M79 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0
M80 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2
M81 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2
M82 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2
```

6.1.3 Phenotype information

Phenotypes were first given for the first trait in the first environment for the first replication, then the second replication and so on. -100.00 is reserved for any missing phenotypes In Table 6.3, one trait (T1) was phenotyped in 4 environments (E1 to E4) with two replications (R1 and R2). The order will be T1E1R1, T1E1R2, T1E2R1, T1E2R2, T1E3R1, T1E3R2, T1E4R1, and T1E14R2. If multiple traits were specified, the order of other traits was the same as the one previousely given.

Table 6.3 Phenotype information in a QTL mapping input file.

```
!** ****** Phenotypic data, -100.00 reserved for missing phenotypic data ************
!Trait name follwoed by phenotypic values for all lines. The order of lines must be
!the same as the order in markertype.
T1E1R1  -3.702  -0.720  -0.002  -3.074  -2.069  -3.073  -4.309  -0.476  -0.250  -0.929  -0.647  -2.066  -3.500  -0.044  -4.033
-4.056
       -6.404  -6.003  -3.320  -3.572  -4.949  -5.257  -2.624  -4.366  -4.252  -3.607  -4.263  -3.588  -0.760  -2.023  -3.200
0.066
        0.085  -3.082  -0.865   0.357  -0.499  -0.060  -3.397  -3.285  -3.060  -3.280  -3.957  -3.893  -0.400  -3.400  -2.668
-3.680
       -2.365  -3.762  -3.066  -3.487  -2.469  -3.050  -2.045  -0.954  -4.500  -2.746  -0.846  -3.054  -0.286  -2.749  -2.230
-0.857
       -0.920  -3.622
T1E1R2  -3.805  -0.206  -0.943  -4.320  -2.504  -3.705  -2.963  -0.697  -0.989  -2.046  -0.747  -2.078  -3.249  -0.202  -3.934
-5.200
       -6.706  -6.269  -3.626  -4.757  -5.755  -5.062  -2.680  -5.500  -3.679  -3.745  -3.866  -2.008  -0.200  -3.366  -3.059
-0.097
       -0.300  -2.890  -0.637  -0.820  -2.833  -3.457  -2.435  -3.307  -2.668  -3.025  -2.592  -3.035  -3.402  -2.685  -2.535
-3.206
       -3.483  -2.799  -3.063  -2.544  -3.698  -2.593  -3.200  -0.702  -2.539  -3.285  -2.320  -3.508   0.603  -2.363  -3.909
-0.688
       -0.930  -2.840
T1E2R1  -2.785  -3.202  -0.806  -4.259  -0.698  -0.934  -3.357  -0.098  -0.805  -0.404  -2.509  -0.986  -3.362  -0.454  -3.388
-4.903
       -6.200  -4.034  -4.433  -3.446  -6.702  -4.399  -4.000  -4.230  -3.629  -3.902  -4.459  -3.200   0.972  -3.423  -3.580
0.503
        0.500  -3.706  -0.363  -0.067  -0.000  -0.567  -3.370  -0.875  -3.374  -4.220  -0.886  -2.203  -2.778  -2.638  -2.405
-3.844
       -2.030  -3.937  -4.746  -2.796  -2.479  -3.405  -2.022  -0.250  -3.006  -2.672  -2.774  -2.772   0.805  -2.943  -3.594
-0.445
       -3.344  -3.830
T1E2R2  -2.988  -2.687  -0.637  -3.043  -2.279  -3.080  -4.400  -0.039  -2.020  -2.320  -0.978  -0.565  -2.660  -0.677  -3.886
-3.607
       -4.302  -5.200  -3.066  -2.890  -5.476  -5.000  -3.068  -6.057  -3.872  -3.865  -4.388  -5.054  -0.037  -2.550  -2.286
0.052
        0.690  -2.590  -0.800  -0.590  -0.078  -0.524  -2.609  -4.399  -2.336  -2.535  -3.200  -2.474  -3.030  -3.060  -3.560
-3.025
       -2.069  -2.490  -3.830  -3.072  -2.738  -2.823  -0.937  -0.470  -3.006  -3.230  -3.635  -3.380  -0.038  -3.000  -0.829
-2.345
       -0.007  -2.353
T1E3R1  -2.600  -2.305  -0.044  -3.427  -3.009  -3.093  -2.453  -0.536  -2.700  -0.855  -0.855  -0.720  -3.304  -0.630  -2.752
-4.656
       -5.795  -6.250  -3.257  -5.645  -4.850  -5.552  -2.558  -6.084  -4.773  -4.208  -3.222  -4.284  -0.006  -2.735  -3.076
0.056
        0.279  -2.757  -0.290  -0.880  -0.969  -2.308  -3.355  -3.490  -3.692  -2.640  -2.432  -3.298  -2.582  -0.490  -3.358
-4.298
       -3.498  -3.799  -2.888  -2.439  -2.706  -4.403  -2.235  -0.000  -3.649  -3.549  -2.989  -3.797   0.303  -0.640  -3.280
-2.025
       -0.045  -3.468
T1E3R2  -4.205  -0.958  -2.054  -3.960  -3.404  -3.099  -4.558   0.000  -0.737  -0.990  -2.025  -2.023  -2.998  -2.098  -3.440
-4.734
       -5.079  -5.522  -2.206  -3.729  -4.940  -6.230  -2.620  -5.407  -4.050  -4.407  -3.480  -4.062  -0.004  -3.208  -2.276
0.694
       -0.007  -2.949  -0.695  -0.390  -2.090  -2.282  -2.863  -3.380  -3.270  -3.090  -3.755  -3.094  -2.435  -2.909  -2.030
-3.088
       -3.026  -3.569  -2.959  -2.974  -4.236  -2.638  -3.299  -0.030  -3.432  -3.668  -3.406  -3.040  -0.066  -2.643  -2.088
-0.576
       -2.030  -0.672
T1E4R1  -3.538  -0.936  -0.905  -0.995  -0.476  -2.939  -4.060  -0.623  -2.008  -2.074  -2.505  -2.349  -2.997  -0.075  -3.703
-4.900
       -5.382  -6.202  -3.302  -4.000  -5.730  -5.660  -2.380  -6.382  -3.590  -4.064  -3.860  -3.807  -0.690  -2.327  -3.698
0.390
        0.350  -2.468  -0.482  -0.059  -0.342  -0.829  -3.030  -3.974  -3.097  -3.502  -2.838  -3.540  -3.409  -3.706  -4.358
-2.478
       -2.508  -0.752  -3.746  -3.626  -3.450  -3.073  -3.009  -0.978  -3.200  -2.035  -3.633  -2.072  -0.200  -2.357  -3.640
-2.059
       -0.387  -4.007
T1E4R2  -3.008  -0.760  -2.093  -2.823  -2.427  -0.044  -2.557   0.032  -2.034  -0.970  -2.043  -0.620  -3.492  -0.002  -3.080
-3.084
       -5.327  -5.290  -4.005  -4.064  -6.084  -5.000  -3.298  -5.034  -4.794  -3.607  -4.649  -4.575  -0.584  -3.780  -3.308
-0.074
        0.992  -2.757  -2.002  -0.204  -2.074  -2.380  -3.894  -3.037  -2.770  -0.305  -4.447  -4.200  -2.846  -2.500  -2.674
-3.080
       -2.608  -4.078  -2.004  -3.363  -3.046  -3.270  -4.038  -0.457  -3.904  -4.020  -3.290  -2.306   0.693  -3.536  -3.583
-0.004
       -0.305  -4.029
```

6.2 Input file for QTL mapping with CSS Lines (*.xls or *.xlsx)

One mapping population for QTL mapping with CSS lines can also be defined in an Excel file with the extension name ‘xls’ or ‘xlsx’. The file should be composed of five sheets: ‘GeneralInfo’ (similar to Table 6.1), ‘Genotype’ (similar to Table 6.2), and ‘Phenotype’ (similar to Table 6.3).

6.3 Setting mapping parameters

The CSL functionality can be initiated by (1) opening input files, or (2) double clicking CSL files listed in the project window, or (3) clicking one CSL file in the display window (Figure 6.1). When functionality CSL is activated, the Display window shows the contents of the current input file, and the Parameter window is for interaction with users (Figure 6.1).

Three mapping methods can be conducted, i.e. single marker analysis (SMA), likelihood ratio test based on stepwise regression for additive QTL (RSTEP-LRT-ADD), likelihood ratio test based on stepwise regression for digenic epistasis (RSTEP-LRT-EPI). The threshold LOD score declaring significant additive QTL or QTL networks can be specified or determined from a number of permutation tests. Once parameters are set for a method, to click  to select the defined method. to click  to unselect a method in the Selected Methods box (Figure 6.1).

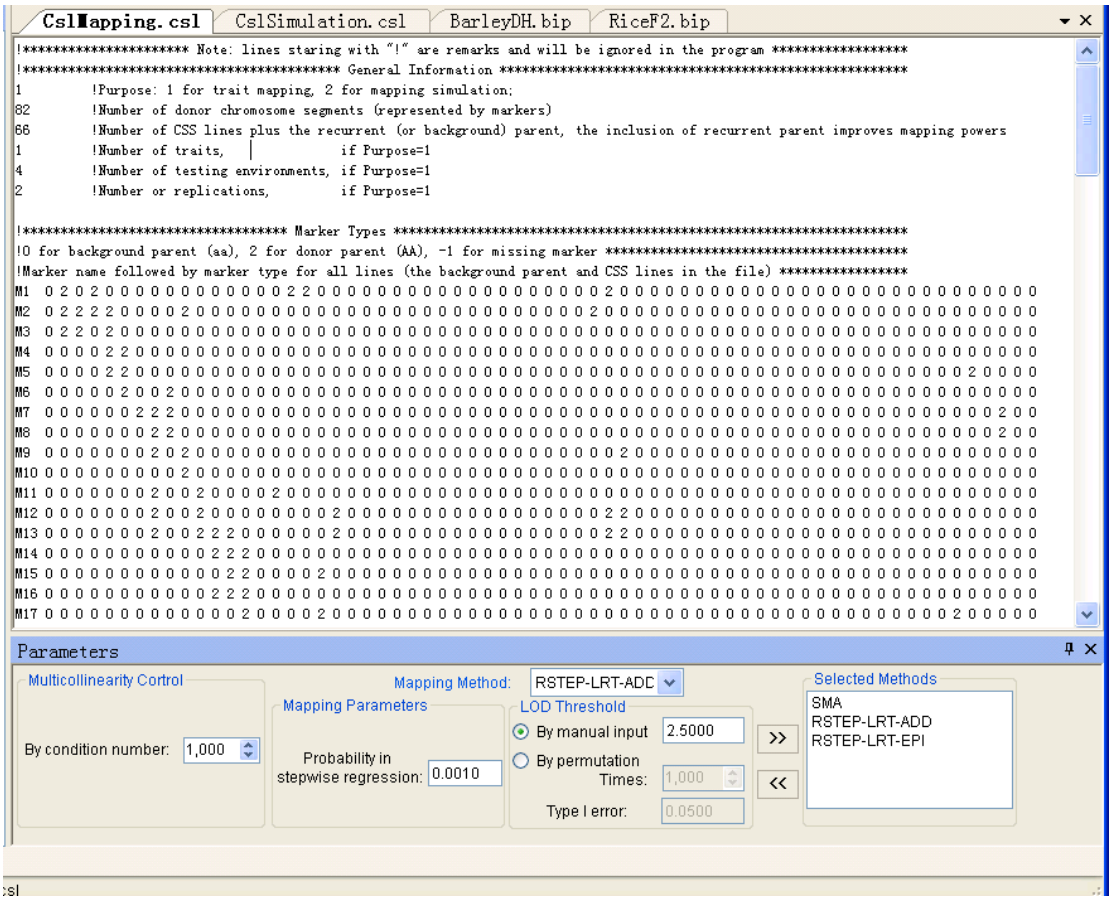

Figure 6.1 Display and Parameter windows in CSL functionality

6.3.1 Handling multi-collinearity

The box on the left in Parameter Window (Figure 6.1) is used to specify a threshold value of condition number for reducing multi-collinearity among marker variables. If a negative number was specified, only duplicate markers will be deleted before QTL mapping. Or the most closely correlated marker will be sequentially deleted until the specifed condition number was reached. Only the remaing marker variables are used in QTL mapping.

6.3.2 Parameters for single marker analysis (Figure 6.2)

- LOD Threshold: the threshold LOD score to declare significant QTL.
  - By manual Input: determine a threshold LOD by user
  - By permutation: determine a threshold LOD by permutation
    - Times: number of permutation tests.
    - Type I error: type I error to determine the LOD threshold from permutation tests.

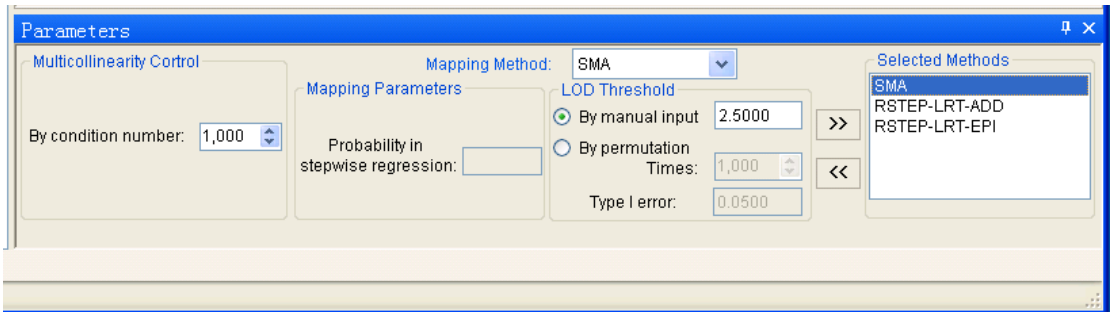

Figure 6.2 Parameters for SMA

6.3.3 Parameters for RSTEP-LRT for additive (Figure 6.3)

- Probability in stepwise regression: the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables (PIN). The largest P-value for removing variables is assumed to be two times of PIN.
- LOD Threshold: the threshold LOD score to declare significant QTL.
    - By manual Input: determine a threshold LOD by user
    - By permutation: determine a threshold LOD by permutation
        - Times: number of permutation tests.
        - Type I error: type I error to determine the LOD threshold from permutation tests.

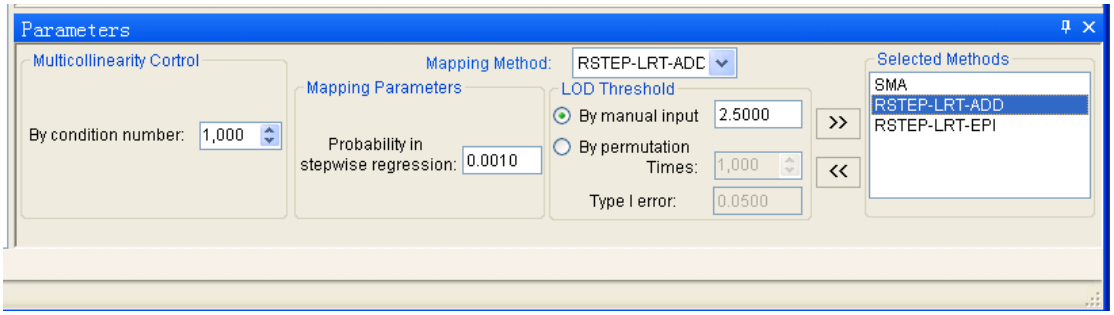

Figure 6.3 Parameters for RSTEP-LRT-ADD

6.3.4 Parameters for RSTEP-LRT for epistasis (Figure 6.4)

- Probability in stepwise regression: the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables (PIN). The largest P-value for removing variables is assumed to be two times of PIN.
- LOD Threshold: the threshold LOD score to declare significant QTL.
    - By manual Input: determine a threshold LOD by user
    - By permutation: determine a threshold LOD by permutation

- Times: number of permutation tests.
- Type I error: type I error to determine the LOD threshold from permutation tests.

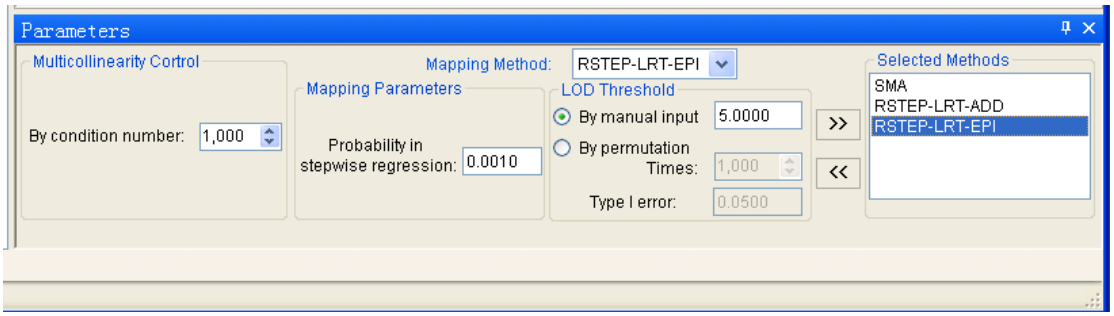

Figure 6.4 Parameters for RSTEP-LRT-EPI

6.4 Outputs

Nine output files are generated from QTL mapping for CSS lines. Three output files were generated from permutation tests for CSS lines.

6.4.1 General information output files

1. STA: Basic statistics of phenotypic datas and results of analysis of variance for each trait (Part 1 in Table 6.4, and Part 2 in Table 6.5)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- n: size of the mapping population.
- Mean: Mean of the phenotypic trait.
- Variance: Variance of the phenotypic trait.
- Std: Standard deviation of the phenotypic trait.
- Skewness: Skewness of the phenotypic trait.
- Kurtosis: Kurtosis of the phenotypic trait.
- Min: Minimum value of the phenotypic trait.
- Max: Maximum value of the phenotypic trait.
- Range: Range of the phenotypic trait.
- W-test: The Shapiro Wilk W-statistic for the test of normality.
- P-Value: P-value of the W-test of normality.

- R-square (%): R-square of ANOVA for the phenotypic trait.
- AdjusedR^2(%): Adjusted R-square of ANOVA for the phenotypic trait.
- Est.Std.Dev.: Estimated standard deviation of the phenotypic trait

- Source: The source of variance.
- DF: The degree of freedom of variance.
- SS: Sum of Squares.
- MS: The mean square of variance
- F: Value of F-test.
- P: P-value of F-test.

Table 6.4 Basic statistics of phenotypic data in the population (STA, Part 1)

TraitID	TraitName	SampleSize	Mean	Variance	STD	Skewness	Kurtosis	Min	Max	Range	W-test	P-value
1	T1E1R1	66	-2.6577	2.2842	1.5113	-0.0071	-0.2035	-6.5550	0.1585	6.7135	0.9428	0.0079
1	T1E2R1	66	-2.5413	2.2788	1.5096	0.2828	-0.3946	-6.0890	0.5950	6.6840	0.9545	0.0402
1	T1E3R1	66	-2.7146	2.2383	1.4961	0.1070	-0.2724	-5.8910	0.3750	6.2660	0.9563	0.0512
1	T1E4R1	66	-2.6975	2.3165	1.5220	0.1798	-0.3499	-5.9070	0.6710	6.5780	0.9581	0.0650

Table 6.5 Analysis of variance for each trait (STA, Part 2, incomplete)

ANOVA for 1th trait
ANOVA for 1th environment

R-square(%)	AdjusedR^2(%)	Est.Std.Dev.	Mean
89.9451	80.0424	0.7092	-2.6577

Source	DF	SS	MS	F	P
Among Groups	65	296.9411	4.5683	9.0830	0.0000
Within Groups	66	33.1950	0.5030		
Total	131	330.1361			

……

2. COE: Coefficient matrix between markers (Table 6.6)

- MarkerID: Marker ID represented by an integer number.
- MarkerName: Marker name, same as given in the CSL input file.

Table 6.6 Correlation coefficient matrix between markers (COE, incomplete)

MarkerID	MarkerName	1	3	4	6	……
1	M1	1.0000	0.2124	-0.0506	-0.0506	……
3	M3	0.2124	1.0000	0.3858	-0.0386	……
4	M4	-0.0506	0.3858	1.0000	0.4844	……
6	M6	-0.0506	-0.0386	0.4844	1.0000	……

……

3. STP: Results from stepwise regression (Table 6.7)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Environment: Environment ID represented by an integer number.
- Numbers after Environment are markers retained in the stepwise regression.
- Intercept: Intercept of the stepwise regression.
- R-square: Phenotypic variation explained by the final regression model.

Table 6.7 information in STP file (incomplete)

TraitID	TraitName	Environment	3	8	11	17	21	26	31	38	41	44	Intercept	R-square
1	T1E1R1	1	0.98	0.95	0.69	1.20	-1.44	-1.12	-0.35	1.41	1.21	0.67	0.23	87.05

……

6.4.2 Results from all markers after handling multi-collinearity

1. RSM: Results from single marker analysis (Table 6.8)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Environment: Environment ID represented by an integer number.
- MarkerID: Marker ID represented by an integer number.
- MarkerName: Marker name, same as given in the CSL input file.
- LOD: LOD score calculated from single marker analysis.
- PVE (%): Phenotypic variation expelained by the marker.
- EstA: Estimated additive effect of the marker.
- M (QQ): Mean value of the QTL genotype QQ (the genotype of donor parent).
- M (qq): Mean value of the QTL genotype qq (the genotype of background parent).

Table 6.8 information in RSM file (incomplete)

TraitID	TraitName	Environment	MarkerID	MarkerName	LOD	PVE(%)	EstA	M(QQ)	M(qq)
1	T1E1R1	1	1	M1	0.6040	3.6611	-0.5757	-3.7219	-2.5705
1	T1E1R1	1	3	M3	0.7818	4.7102	0.8296	-1.0740	-2.7331
1	T1E1R1	1	4	M4	0.0065	0.0400	-0.0928	-2.8377	-2.6521
1	T1E1R1	1	6	M6	0.0853	0.5262	0.3369	-2.0043	-2.6781
1	T1E1R1	1	8	M8	1.2656	7.4984	1.0467	-0.6595	-2.7528
1	T1E1R1	1	9	M9	0.3032	1.8569	0.5209	-1.6633	-2.7050
1	T1E1R1	1	10	M10	0.1348	0.8308	0.5941	-1.4875	-2.6757
1	T1E1R1	1	11	M11	0.1440	0.8869	0.3600	-1.9705	-2.6904
1	T1E1R1	1	12	M12	0.2109	1.2961	0.3425	-2.0245	-2.7096
1	T1E1R1	1	14	M14	0.1961	1.2055	0.4197	-1.8565	-2.6958
1	T1E1R1	1	17	M17	0.7108	4.2925	0.7919	-1.1458	-2.7297
1	T1E1R1	1	18	M18	0.0154	0.0951	0.1179	-2.4327	-2.6684
1	T1E1R1	1	19	M19	0.3856	2.3555	-1.0003	-4.6280	-2.6274
1	T1E1R1	1	21	M21	3.6609	19.9992	-1.3456	-5.1450	-2.4538
1	T1E1R1	1	23	M23	2.0259	11.6951	-1.1411	-4.8016	-2.5194
1	T1E1R1	1	25	M25	0.2243	1.3776	-0.7650	-4.1645	-2.6345
1	T1E1R1	1	26	M26	1.4111	8.3188	-1.3396	-5.2558	-2.5765
1	T1E1R1	1	28	M28	0.2587	1.5871	-0.5851	-3.7925	-2.6222
1	T1E1R1	1	30	M30	0.7321	4.4181	-0.8034	-4.1915	-2.5847

……

2. RAD: Results from RSTEP-LRT-ADD, same format as RSM

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Environment: Environment ID represented by an integer number.
- MarkerID: Marker ID represented by an integer number.
- MarkerName: Marker name, same as given in the CSL input file.
- LOD: LOD score calculated from single marker analysis.
- PVE (%): Phenotypic variation expelained by the marker.
- EstA: Estimated additive effect of the marker.
- M (QQ): Mean value of the QTL genotype QQ (the genotype of donor parent).
- M (qq): Mean value of the QTL genotype qq (the genotype of background parent).

3. REP: Intercations between markers (Part 1 in Table 6.9, and Part 2 in Table 6.10)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.

- Environment: Environment ID represented by an integer number.
- MarkerID1: Marker ID of the first marker represented by an integer number.
- MarkerName1: Marker name of the first marker, same as given in the CSL input file.
- MarkerID2: Marker ID of the second marker represented by an integer number.
- MarkerName2: Marker name of the second marker, same as given in the CSL input file.
- LOD: LOD score calculated from epistatic mapping.
- PVE (%): Phenotypic variation expelained by the marker.

- EstA1: Estimated additive effect of the first QTL.
- EstA2: Estimated additive effect of the second QTL.
- EstAA: Estimated additive by additive effect of QTL.
- M (Q1Q1Q2Q2): Mean value of the QTL genotype Q1Q1Q2Q2. Q1 and q1 are the two alleles at the first marker, and Q2 and q2 are the two alleles at the second marker. Q is from donor parent, and q is from background parent.
- M (Q1Q1q2q2): Mean value of the QTL genotype Q1Q1q2q2. Q1 and q1 are the two alleles at the first marker, and Q2 and q2 are the two alleles at the second marker. Q is from donor parent, and q is from background parent.
- M (q1q1Q2Q2): Mean value of the QTL genotype q1q1Q2Q2. Q1 and q1 are the two alleles at the first marker, and Q2 and q2 are the two alleles at the second marker. Q is from donor parent, and q is from background parent.
- M (q1q1q2q2): Mean value of the QTL genotype q1q1q2q2. Q1 and q1 are the two alleles at the first marker, and Q2 and q2 are the two alleles at the second marker. Q is from donor parent, and q is from background parent.

Table 6.9 information in REP file (Part 1, incomplete)

TraitID	TraitName	Environment	MarkerID1	MarkerName1	MarkerID2	MarkerName2	LOD	PVE(%)
1	T1E1R1	1	1	M1	3	M3	1.7972	8.7124
1	T1E1R1	1	1	M1	12	M12	0.3582	1.6233
1	T1E1R1	1	1	M1	21	M21	3.6632	19.3061
……								

Table 6.10 information in REP file (Part 2, incomplete)

EstA1	EstA2	EstAA	M(Q1Q1Q2Q2)	M(Q1Q1q2q2)	M(q1q1Q2Q2)	M(q1q1q2q2)
0.0159	1.2464	0.3005	1.9041	-1.1897	1.2713	-0.6204
-0.3926	-0.3993	-0.4768	-1.6776	0.0746	0.0612	-0.0937
-0.0801	-1.1614	0.2035	-1.0159	0.8999	-1.2628	1.4671
……						

6.4.3 Results files from significant QTL

1. QSM: All significant markers from single marker analysis (the format of QSM file is similar to Table 6.8)
2. QAD: All significant QTL from one-dimensional scanning for QTL with additive effects under additive mapping using RSTEP-LRT (the format of QAD file is

similar to Table 6.8)

3. QEP: All significant peaks from two-dimensional scanning for QTL networks (the format of QEP file is similar to Table 6.9, 6.10)

6.4.4 Results files from permutation tests

1. TSM: the maximum LOD score from single marker analysis across all the markers for each permutation test (Table 6.11)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Environment: Environment ID represented by an integer number.
- Times: Times in permutation tests. The threshold LOD was given after all permutation tests.
- MaxLOD: Maximum LOD score in the permutation test.

Table 6.11 information in TSM file (incomplete)

TraitID	TraitName	Environment	Times	MaxLOD
1	T1E1R1	1	1	2.1992
1	T1E1R1	1	2	1.2005
……				
1	T1E1R1	1	99	1.9870
1	T1E1R1	1	100	1.1540
1	T1E1R1	1	Threshold	2.0721

2. TAD: the maximum LOD score from additive mapping across all the markers for each permutation test (the format of TAD file is similar to Table 6.11)
3. TEP: the maximum LOD score from epistasis mapping across all the markers for each permutation test (the format of TEP file is similar to Table 6.11)

6.5 Figures

Bar graphs are avaible for SMA and RSTEP-LRT-ADD for the CSL functionality. Figures can be initiated by chosing the Figures menu (Figure 6.5).

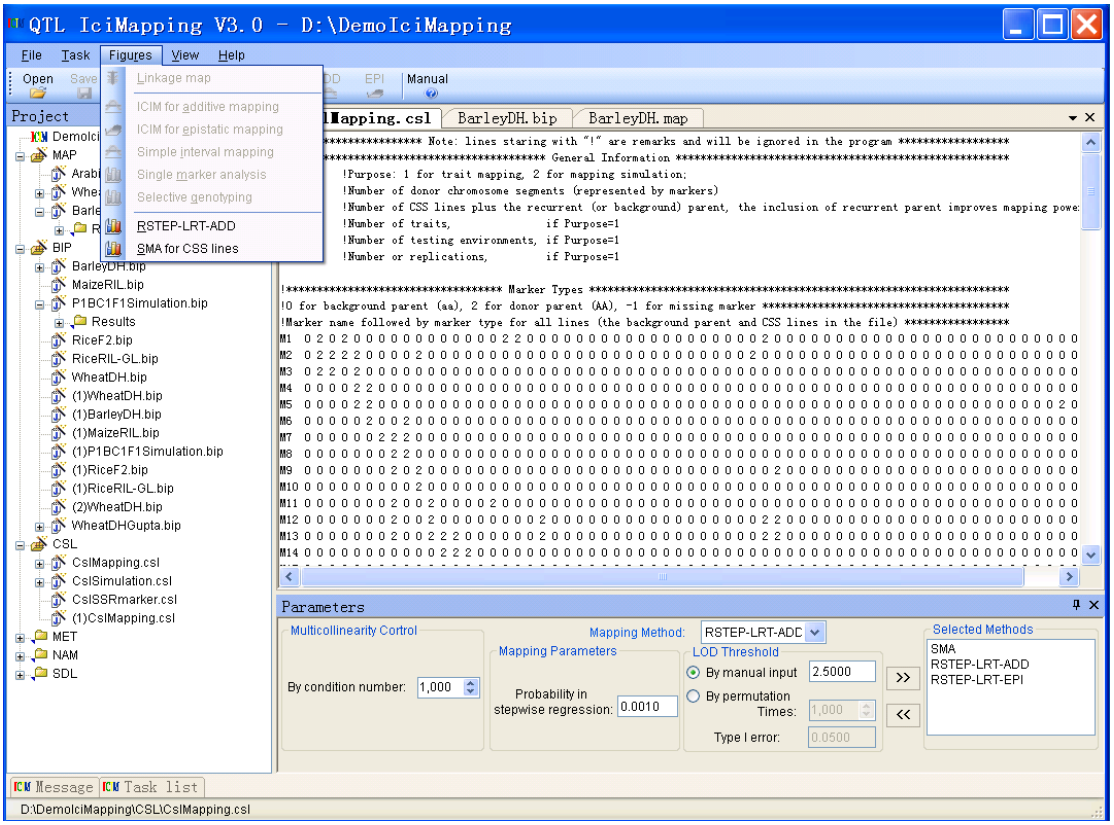

Figure 6.5 Figures menu for the CSL functionality

6.5.1 RSTEP –LRT-ADD (Figure 6.6)

Click RSTEP -LRT-ADD in the Figures menu to draw graphs. A new window with the title “[Graph] RSTEP -LRT-ADD - …” will be open to show the bar graph (Figure 6.6). Similar tool bars as ICIM-ADD are available to change the figure format.

6.5.2 Figures of single marker analysis (Figure 6.7)

Click RSTEP -LRT-ADD in the Figures menu to draw graphs. A new window with the title “[Graph] RSTEP -LRT-ADD - …” will be open to show the bar graph (Figure 6.6). Similar tool bars as ICIM-ADD are available to change the figure format.

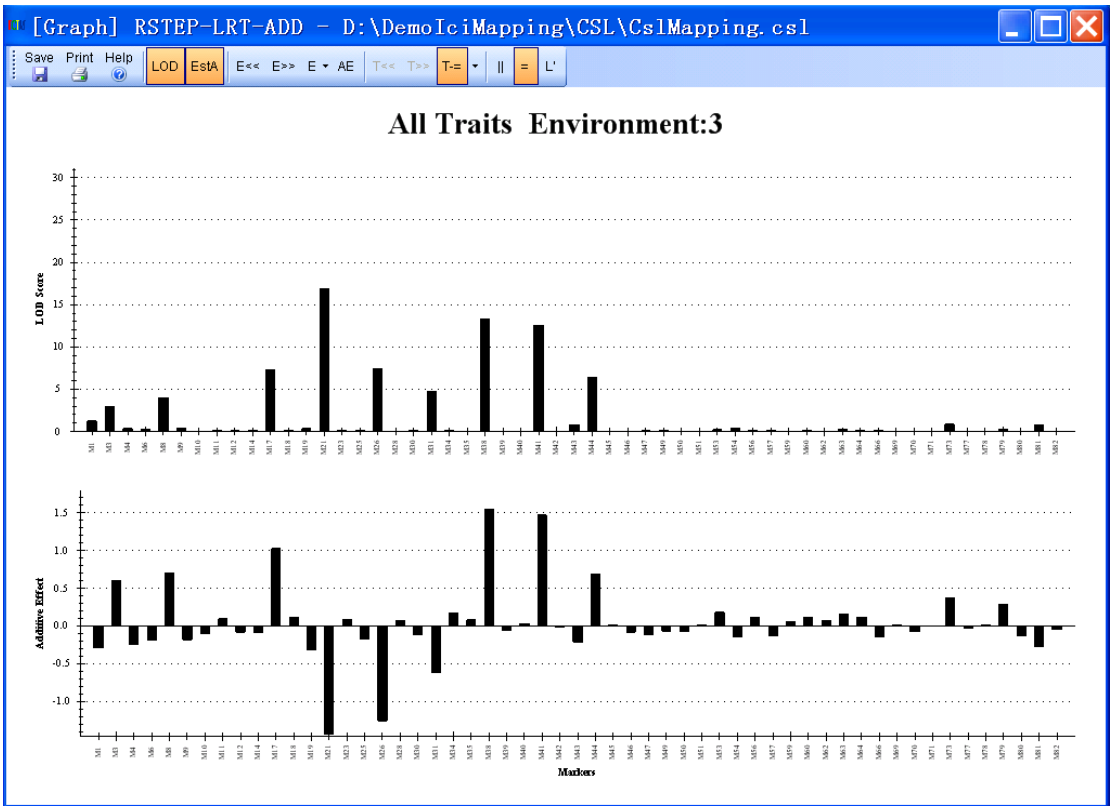

Figure 6.6 Figures of RSTEP-LRT-ADD

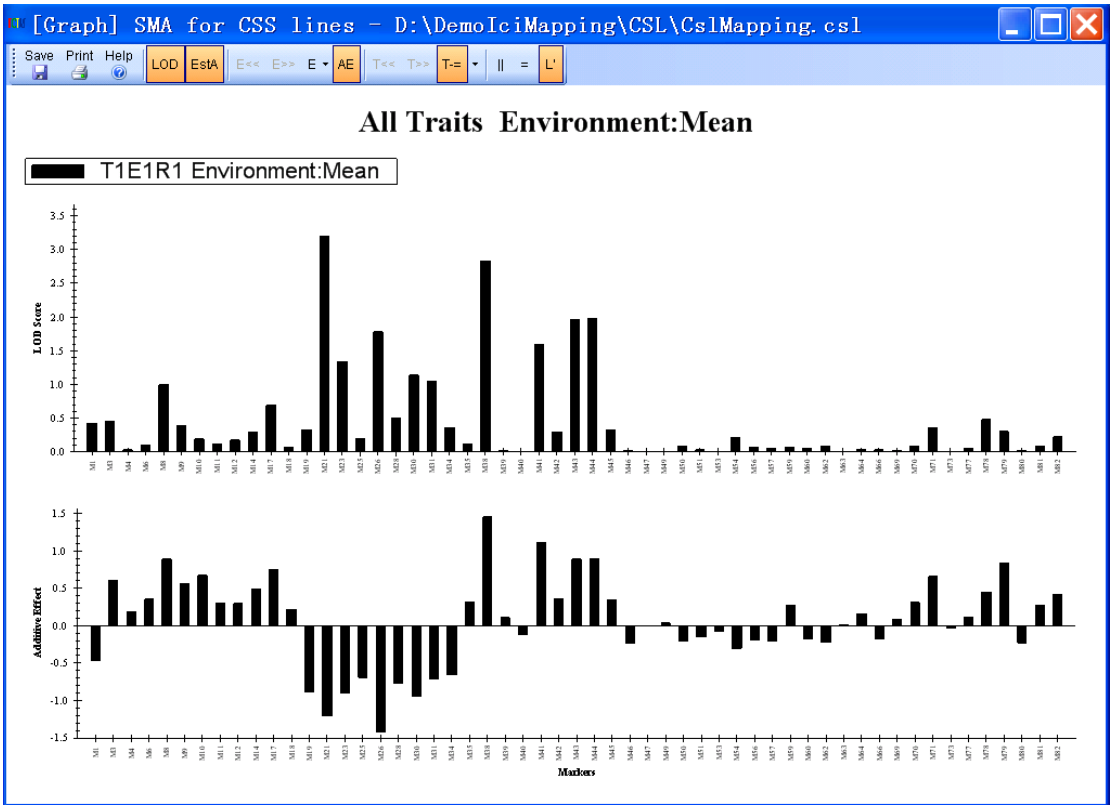

Figure 6.7 Figures of single marker analysis

Chapter 7. Power analysis with CSS lines (CSL)

By using QTL IciMapping, one can also conduct power analysis for a set of predefined QTL so as to compare the efficiency of different mappingmethods. Please note, we only simulated phenotypic data in CSL. Marker type has to be given. Tables 7.1-7.3 give a working example for 66 CSS lines for power analysis.

7.1 Input file for power analysis with CSS Lines (*.csl)

7.1.1 General population information

Three parameters were used for the general information describing the power analysis with CSS lines (Table 7.1).

- Indicator: let IciMapping know if a mapping study or power simulation will be conducted. Indicator 2 has to be assigned for any power simulation studies (Table 7.1).
  - 1 for a mapping study.
  - 2 for a power simulation.
- Number of Donor Chromosome Segments: specify the number of donor chromosome segments in these CSS lines.
- Number of CSS Lines: number of CSS lines in the mapping population. It has been proved that the inclusion of the background will reduce the marker collinearity and therefore improve the mapping power. For example, if 65 CSS lines and their background parent were used in QTL mapping, the number of 66 has to be specified.

Table 7.1 General population information in a power analysis input file.

```
!********* ********* Input file for QTL mapping with CSS lines ********************
!Lines starting with '!' are remarks and will be ignored when read.
2        !Purpose: 1 for trait mapping, 2 for mapping simulation;
82       !Number of donor chromosome segments (represented by markers)
66       !Number of CSS lines plus the recurrent (or background) parent
```

7.1.2 Marker type information

Marker types are arranged by marker (Table 7.2). The marker types for all individuals on the first marker were given first, then followed by the second marker, and so on. In IciMapping, the marker type 2 was used for the marker type of the donor parent, 0 for the background parent, and -1 for any missing markers. Any missing markers will be assigned to the value 0 in QTL mapping. The number of marker types behind each marker name has to be exact the number of lines.

Table 7.2 Marker type information in a power analysis input file.

```
!****************** Marker type: 0 for background parent (aa), 2 for donor parent (AA), -1 for missing marker *************************
!Marker name followed by marker type for all lines (the kackground parent and CSS lines)
M1  0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M2  0 2 2 2 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M3  0 2 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M4  0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M5  0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0
M6  0 0 0 0 0 2 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M7  0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0
M8  0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0
M9  0 0 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M10 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M11 0 0 0 0 0 0 0 2 0 0 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M12 0 0 0 0 0 0 0 2 0 0 2 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M13 0 0 0 0 0 0 0 2 0 0 2 2 2 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M14 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M15 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M16 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M17 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0
M18 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0
M19 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M20 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M21 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M22 0 2 2 0 0 0 0 0 0 2 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M23 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M24 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M25 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M26 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M27 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M28 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M29 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M30 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M31 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M32 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 2 2 2 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M33 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0
M34 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M35 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M36 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 2 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M37 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M38 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M39 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M40 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M41 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0
M42 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M43 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0
M44 0 0 0 0 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 2 0
M45 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 2 0
M46 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M47 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M48 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 2
M49 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0
M50 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M51 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M52 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M53 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M54 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 2 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M55 0 0 0 0 0 2 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 2 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M56 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M57 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M58 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0
M59 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 2 0 0
M60 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2 0 2 2 0 0 0 0 0 0 0 0 0 0 0
M61 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0
M62 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 2 0 0 0 0 0 0 0 0 0
M63 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0 0 0
M64 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0 0 0 0 0 0
M65 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 2 2 0 0 0 0 0 0 0
M66 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0
M67 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0
M68 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0 0 0
M69 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 2 0 0 0 0 2 0 2 0 0 0 0 0 0 2 0 0 0 0 0 0 0
M70 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0
M71 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0 0 0
M72 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0 0
M73 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 0 0
M74 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0
M75 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0
M76 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 2 0 0
M77 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 2 0 2 2 2 0 0 0 0 0 0 2 0 0 0 2 2
M78 0 0 0 0 0 0 2 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 2 0 0 0 2 2
M79 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0
M80 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2
M81 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2
M82 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2
```

7.1.3 Predefined QTL information

A number of QTL have to be defined before conducting any power simulation studies. In Table 7.3, the number of QTL for a trait of interest was 10, the number of simulation runs was 100, and the heritability in the broad sense was 0.80. The ten QTL were located at chromosome segments represented by markers 2, 12, 23, and so on, and their additive effects were 2.10, -1.80, 3.09, and so on.

Table 7.3 QTL in

```
********** QTL information ***********************************************************
!Row 1: NumQTL   NumSimulations   Heritability
```

```
!Row 2: WhichMarkers harbor these QTL
!Row 3: QTL additive effects
10     100     0.80
2      12      23      24      35      46      47      58      60      70
2.10   -1.80    3.09   -1.00    1.10   -1.60    1.30   -2.00    1.70   -1.23
```

7.2 Input file for power analysis with CSS Lines (*.xls or *.xlsx)

One mapping population for power analysis with CSS lines can also be defined in an Excel file with the extension name ‘xls’ or ‘xlsx’. The file should be composed of five sheets: ‘GeneralInfo’ (similar to Table 7.1), ‘Genotype’ (similar to Table 7.2), and ‘QTLInfo’ (similar to Table 7.3).

7.3 Setting mapping parameters

The CSL functionality can be initiated by (1) opening input files, or (2) double clicking CSL files listed in the project window, or (3) clicking one CSL file in the display window (Figure 7.1). When functionality CSL is activated, the Display window shows the contents of the current input file, and the Parameter window is for interaction with users (Figure 7.1).

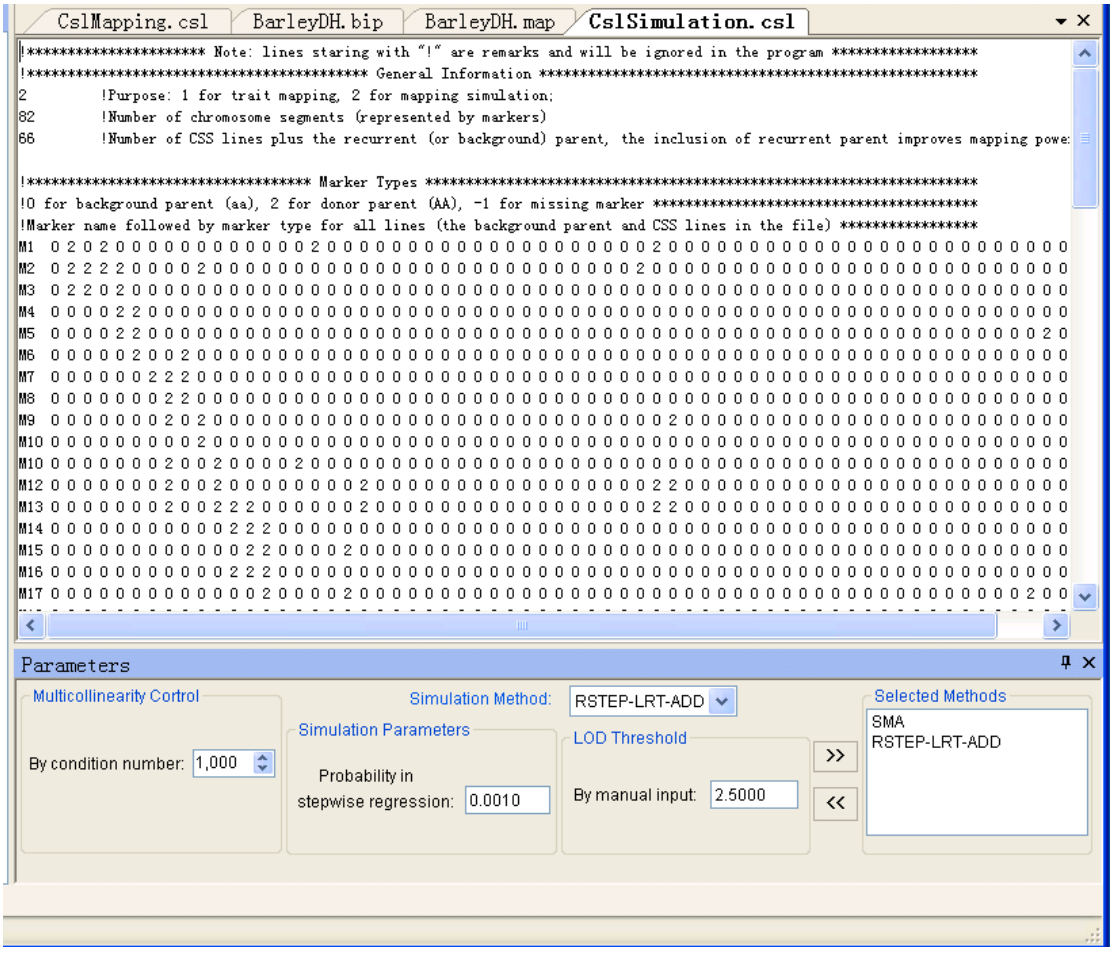

Figure 7.1 Display and Parameter windows in CSLsimulation functionality

Two mapping methods can be conducted, i.e. single marker analysis (SMA), and likelihood ratio test based on stepwise regression for additive QTL (RSTEP-LRT-ADD). The threshold LOD score declaring significant additive QTL or QTL networks has to be specified. Once parameters are set for a method, to click  to select the defined method. to click  to unselect a method in the Selected Methods box (Figure 7.1).

7.3.1 Handling multi-collinearity

The box on the left in Parameter Window (Figure 7.1) is used to specify a threshold value of condition number for reducing multi-collinearity among marker variables. If a negative number was specified, only duplicate markers will be deleted before QTL mapping. Or the most closely correlated marker will be sequentially deleted until the specifed condition number was reached. Only the remaing marker variables are used in QTL mapping.

7.3.2 Parameters for single marker analysis (Figure 7.2)

- LOD Threshold: the threshold LOD score to declare significant QTL.

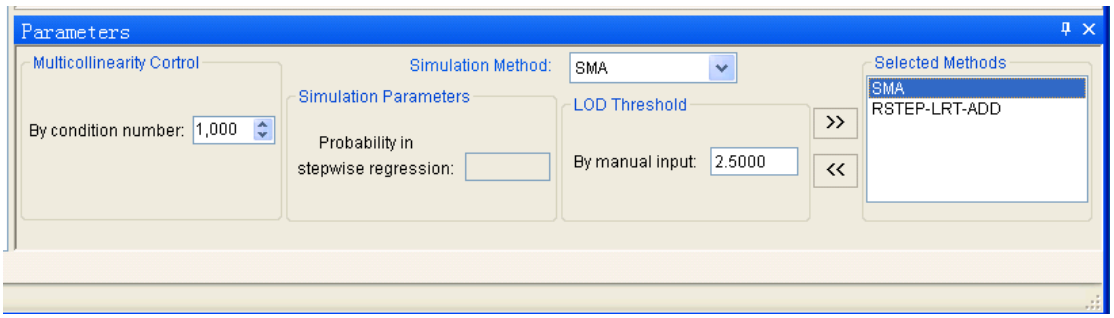

Figure 7.2 Parameters for SMA

7.3.3 Parameters for RSTEP-LRT for additive (Figure 6.3)

- Probability in stepwise regression: the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables (PIN). The largest P-value for removing variables is assumed to be two times of PIN.
- LOD Threshold: the threshold LOD score to declare significant QTL.

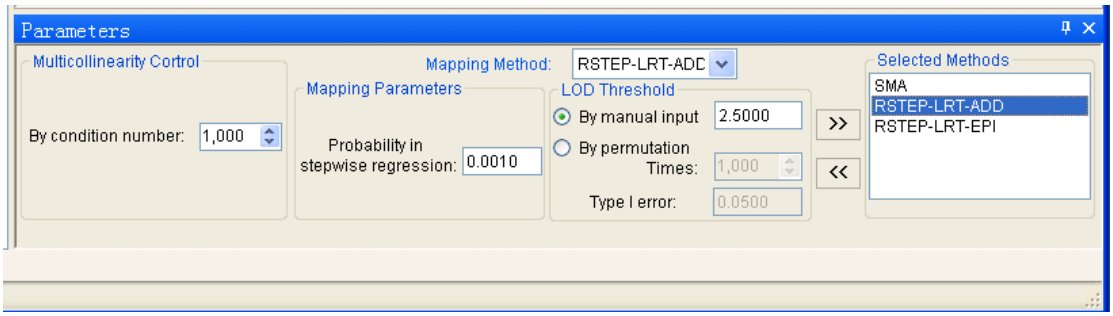

Figure 6.3 Parameters for RSTEP-LRT-ADD

7.4 Outputs

Four output files were generated from power simulation for CSS lines.

7.4.1 General files

1. COE: Coefficient matrix between markers (*incomplete*)

Table 7.4 Correlation coefficient matrix between markers (COE)

MarkerID	MarkerName	1	3	4	6	……
1	M1	1.0000	0.2494	-0.0449	-0.0449	……
3	M3	0.2494	1.0000	0.3858	-0.0386	……
4	M4	-0.0449	0.3858	1.0000	0.4844	……
6	M6	-0.0449	-0.0386	0.4844	1.0000	……
……						

2. STP: Results from stepwise regression (Table 7.5)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the BIP input file.
- Environment: Environment ID represented by an integer number.
- Numbers after Environment are markers retained in the stepwise regression.
- Intercept: Intercept of the stepwise regression.
- R-square: Phenotypic variation explained by the final regression model.

Table 7.5 information in STP file (incomplete)

TraitID	TraitName	Environment	3	12	23	Intercept	R-square
1	SimulatedTrait	1	2.3264	-2.5045	1.8665	1.6398	48.9854
……							

7.4.2 Result files from power analysis

The following files are only for power analysis.

1. PSM: Powers analysis for single marker analysis (Part 1 in Table 7.6, and Part 2 in

7.7)

- Simulation: To represent the times in simulation.
- Numbers after Simulation are markers retained after De-multicollinearity process and the estimated additive effects of these markers.
- MeanEstALL: Mean values of additive effects of markers in all simulations.
- MeanEstAdd: Mean values of additive effects of markers when QTL is detected.
- MeanLOD: Mean values of LOD scores in all simulations.
- Power (%): Detection power of QTL.
- TrueAdd: True additive effects of markers.
- PGVE (%): Genotypic variation expelained by QTL at the marker.
- PPVE (%): Phenotypic variation expelained by QTL at the marker.

Table 7.6 information in PSM file (Part 1, incomplete)

Simulation	1	3	4	6	8 ……
1	1.7430	2.5472	0.4192	-0.8159	-0.6256 ……
2	0.9594	1.9515	0.3691	-1.2868	-0.5552 ……
……					

Table 7.7 information in PSM file (Part 2, incomplete)

Simulation	1	3	4	6	8 ……
……					
MeanEstAll	0.0000	2.4461	0.0000	0.0000	0.0000 ……
MeanEstAdd	0.0000	2.7147	0.0000	0.0000	0.0000 ……
MeanLOD	0.7057	2.0712	0.1149	0.3509	0.2404 ……
Power(%)	0.0000	50.0000	0.0000	0.0000	0.0000 ……
TrueAdd	0.0000	0.0000	0.0000	0.0000	0.0000 ……
PGVE(%)	0.0000	0.0000	0.0000	0.0000	0.0000 ……
PPVE(%)	0.0000	0.0000	0.0000	0.0000	0.0000 ……
PhenoVar	6.4283				
GenoVar	8.0353				

2. PAD: Powers analysis for additive mapping (the format of Part 1 in PAD file is similar to Table 7.6, and the format of Part 2 is similar to Table 7.7)

Chapter 8. QTL by Environment Interactions for Multi-Environment Trials (MET)

8.1 Input file for QTL by environment interactions (*.met)

8.1.1 General information of the mapping population

Seven parameters were used for the general information defining a linkage mapping population (Table 8.1).

- Population Type: describe the type of the population. At present, QTL IciMapping can conduct QTL mapping for twenty populations derived from two parental lines for multi-environmental trials (Figure 1.1). Assuming F1 = P1 x P2, the 20 biparental populations are:
  - 1. P1BC1F1: the backcross population where the first parent (P1) is used as the recurrent.
  - 2. P2BC1F1: the backcross population where the second parent (P2) is used as the recurrent.
  - 3. F1DH: doubled haploids derived from F1.
  - 4. RIL: recombination inbred lines derived from repeated selfing since F1 generation.
  - 5. P1BC1RIL: recombination inbred lines derived from the backcross population where the first parent is used as the recurrent.
  - 6. P2BC1RIL: recombination inbred lines derived from the backcross population where the second parent is used as the recurrent.
  - 7. F2: the selfing generation of F1.
  - 8. F3: the selfing generation of F2.
  - 9. P1BC2F1: the second backcrossing where P1 is used as the recurrent parent.
  - 10. P2BC2F1: the second backcrossing where P2 is used as the recurrent parent.
  - 11. P1BC2RIL: recombination inred lines through the repeated selfing of P1BC2F1.
  - 12. P2BC2RIL: recombination inred lines through the repeated selfing of P2BC2F1.
  - 13. P1BC1F2: the selfing generation of P1BC1F1.
  - 14. P2BC1F2: the selfing generation of P2BC1F1.
  - 15. P1BC2F2: the selfing generation of P1BC2F1.
  - 16. P2BC2F2: the selfing generation of P2BC2F1.
  - 17. P1BC1DH: P1BC1F1-derived doubled haploids.
  - 18. P2BC1DH: P2BC1F1-derived doubled haploids.
  - 19. P1BC2DH: P1BC2F1-derived doubled haploids.
  - 20. P2BC2DH: P2BC2F1-derived doubled haploids.

- Mapping Function: specify the mapping function which will be used to transfer recombination frequency to mapping distance, or from mapping distance to recombination frequency.
  - 1 for Kosombi mapping function.
  - 2 for Haldane mapping function.
  - 3 for Morgan mapping function.

- Marker Space Type: specify whether the markers on a chromosome (or linkage group) are defined by positions or marker intervals.
  - 1 for intervals, i.e. the number behind a marker is the distance of the marker to its next marker. 0 is normally given for the last marker on a chromosome or a linkage group.
  - 2 for positions, i.e. the number behind each marker is the position of the marker on the chromosome or the linkage group.

- Marker Space Unit: specify the unit used in marker linkage group.
  - 1 for centi-Morgan (cM).
  - 2 for Morgan (M). 1 M = 100 cM.

- Number of Chromosomes: specify the number of chromosomes (or linkage groups) in the mapping population.

- Population Size: number of individuals in the mapping population.

- Number of Environments: number of environments phenotyped in the mapping population.

Table 8.1 General information in a QTL mapping input file

```
!***** Note: lines staring with "!" are remarks and will be ignored in the program***
!***** General Information ********************************************************
!Assuming F1 = P1 x P2, populations available in QTL IciMapping are:
!  1, P1BC1F1 = P1 x F1, the first backcrossing where P1 is used as the recurrent parent;
!  2, P2BC1F1 = P2 x F1, the first backcrossing where P2 is used as the recurrent parent;
!  3, F1DH, F1-derived doubled haploids;
!  4, RIL or F1RIL, recombination inbred lines through the repeated selfing of F1;
!  5, P1BC1RIL, recombination inbred lines through the repeated selfing of P1BC1F1;
!  6, P2BC1RIL, recombination inbred lines through the repeated selfing of P2BC1F1;
!  7, F2, the selfing generation of F1;
!  8, F3, the selfing generation of F2;
!  9, P1BC2F1, the second backcrossing where P1 is used as the recurrent parent;
! 10, P2BC2F1, the second backcrossing where P2 is used as the recurrent parent;
! 11, P1BC2RIL, recombination inbred lines through the repeated selfing of P1BC2F1;
! 12, P2BC2RIL, recombination inbred lines through the repeated selfing of P2BC2F1;
! 13, P1BC1F2, the selfing generation of P1BC1F1;
! 14, P2BC1F2, the selfing generation of P2BC1F1;
! 15, P1BC2F2, the selfing generation of P1BC2F1;
! 16, P2BC2F2, the selfing generation of P2BC2F1;
! 17, P1BC1DH, P1BC1F1-derived doubled haploids;
! 18, P2BC1DH, P2BC1F1-derived doubled haploids;
! 19, P1BC2DH, P1BC2F1-derived doubled haploids;
! 20, P2BC2DH, P2BC2F1-derived doubled haploids;
   4     !Mapping Population (see remarks above)
   2     !Mapping Function (1 for Kosambi; 2 for Haldane; 3 for Morgan)
   2     !Marker Space Type (1 for intervals; 2 for positions)
   1     !Marker Space Unit(1 for centiMorgans; 2 for Morgan)
  10     !Number of Chromosomes (or Linkage Group)
  194    !Population size of the mapping population
```

```
7     !Number of environments
```

8.1.2 Linkage group or chromosome information

The name of each chromosome and the number of markers on the chromosome were specified first (Table 8.2), followed by the definition of each chromosome (Table 8.3). Each chromosome was defined by all markers on it and all marker positions.

Table 8.2 Linkage group information in a QTL mapping input file for MET

```
!***********************Information for Chromosomes and Markers***********************
!Chromosome     NumMarkers in each chromosome
-Ch1                    61
-Ch2                    44
-Ch3                    43
-Ch4                    38
-Ch5                    42
-Ch6                    28
-Ch7                    25
-Ch8                    36
-Ch9                    30
-Ch10                   28
```

Table 8.3 Definition of each chromosome (incomplete)

```
!Linkage map (Marker name followed by position or the interval length)
L00411   1   0
L00569   1   3.7
L00068   1   9.7
L01003   1   13.4
L00196   1   15.6
L00609   1   17.9
L00181   1   20.1
L00828   1   24.4
L00454   1   29.7
L01110   1   31.7
L00890   1   33.9
L00254   1   37.8
L00929   1   43.2
L01185   1   47.3
L00659   1   51.7
L01075   1   54
L00604   1   56.5
L01015   1   59
L00174   1   61.6
L00116   1   64.9
L00707   1   69.1
L00830   1   72
L00120   1   74.7
L00410   1   79.9
L00388   1   82.4
L00789c  1   84.6
L00916   1   86.6
L00356   1   88.6
L00708   1   91.4
L00003   1   94.9
L00585   1   97.7
L00294   1   108.4
L00755   1   115
L00368   1   120.3
L00780   1   123.7
L00795   1   125.9
L00048   1   129.8
L00300   1   132.9
L01115   1   135.2
L00736   1   137.6
L00240   1   140.2
L00750   1   146
```

```
L00603c  1   149.7
L00082   1   151.8
L00465   1   155.6
L00734   1   157.6
L01174   1   162.2
L00699   1   164.6
L00165   1   167.8
L00588   1   171.4
L01039   1   174.6
L01149   1   176.9
L00210   1   179.1
L00742   1   181.3
L01182   1   185
L00553   1   188.2
L00222   1   191.5
L00295   1   193.8
L00158   1   196.5
L00341   1   199.4
L00503   1   202
……
L01150   10  33.3
L00721   10  35.8
L00509   10  38.6
L00227   10  40.6
L00166   10  42.9
L01065   10  46.7
L00781   10  48.8
L01041   10  52.2
L00614   10  56.1
L00912   10  58.4
L00446   10  61.6
L00047   10  64.5
L00539   10  69.2
L00843   10  75.4
L00186   10  80.8
L00381   10  83.2
L00578   10  85.3
L01062   10  91
L00170   10  93.7
L00665   10  101.9
```

8.1.3 Marker type information

Marker types are arranged by the ordered markers defined in Table 8.3. That is, the marker types for all individuals on the first marker were given first, then followed by the second marker, and so on (Table 8.4). The marker name in this part has to be the same as that specified in Table 8.3. In IciMapping, 2 was used to represent the marker type of the first parent (P1), 0 for the second parent (P2), 1 for the F1 marker type, and -1 for any missing markers. Any missing markers will be assigned values based on the types of their neighboring markers. The number of marker types behind each marker name has to be exact the population size.

Table 8.4 Marker type information in a QTL mapping input file (incomplete)

```
!**********************************Marker Type****************************************
!Marker type: 2 for P1; 1 for F1; 0 for P2; BC1=F1xP1; BC2=F1xP2; -1 for missing markers
L00411   -1  2   0   0   0   2   2   0   2   0   0   2   0   2   -1  2   0   0
     0   -1  2   0   2   2   2   0   0   0   2   2   2   2   2   0   -1  2   2
     -1  2   2   0   2   2   0   2   -1  -1  2   2   0   -1  0   0   -1  2   2
     2   0   0   2   2   2   -1  0   0   -1  2   0   2   2   2   0   2   0   0
     2   2   2   2   0   -1  0   -1  2   2   2   0   2   -1  2   2   0   0   0
     2   0   2   0   2   0   -1  0   0   2   2   0   0   0   2   2   2   0   2
     2   0   0   0   2   0   0   2   0   2   0   2   2   0   0   2   0   0   2
     0   0   0   2   0   2   0   0   0   2   2   0   2   0   0   0   2   0   0
     2   -1  0   0   2   -1  2   0   0   2   2   2   0   2   0   0   2   2   2
     0   0   0   0   2   0   0   2   2   0   0   2   0   0   0   2   0   2   2
     0   0   0   2   0
```

```
L00569   0   2   0   0   0   2   2   0   2   0   0   2   0   2   2   2   0   0
     0   -1  2   0   2   0   2   2   0   0   2   2   2   2   2   0   -1  2   2
     -1  2   2   -1  2   2   0   2   -1  2   2   2   0   -1  0   0   0   2   2
     2   0   0   2   2   2   -1  0   0   -1  2   0   2   2   2   -1  2   0   0
     2   2   2   2   0   -1  0   2   2   2   -1  0   2   -1  0   2   0   0   0
     0   0   2   2   2   0   2   0   0   2   2   0   0   0   2   2   2   0   2
     2   0   0   0   2   0   0   2   0   2   0   2   2   0   0   2   0   0   2
     0   0   0   2   0   0   0   0   0   2   2   0   2   0   0   0   2   0   0
     2   -1  0   0   2   0   2   0   0   2   2   2   0   2   0   0   2   2   2
     0   0   0   0   0   0   0   2   2   0   0   2   0   0   0   2   2   2   2
     0   0   0   0   0
L00068   0   2   0   0   0   2   2   0   2   0   0   2   0   2   2   2   0   -1
     0   0   2   0   2   0   0   2   0   0   2   2   2   2   -1  0   -1  -1  0
     -1  2   2   -1  2   2   0   2   -1  2   2   2   0   -1  0   0   0   2   0
     2   2   0   2   0   2   -1  0   0   -1  2   0   2   2   2   -1  2   0   0
     2   2   2   2   2   -1  0   0   0   2   -1  2   2   2   0   -1  0   0   -1
     -1  0   0   2   2   0   2   0   0   2   2   0   2   0   2   2   2   0   2
     2   0   0   0   2   2   0   2   0   2   0   2   2   0   0   2   0   -1  2
     0   0   0   -1  2   -1  0   0   0   0   2   0   2   0   0   0   2   2   0
     2   -1  0   0   2   0   0   0   0   2   2   2   0   2   0   0   2   2   2
     0   0   0   0   0   -1  0   2   2   0   0   0   0   2   0   0   2   2   2
     0   0   0   0   0
……
```

8.1.4 Phenotyp information

Phenotypes were first given for the first envrionment, then for the second envrionment and so on. -100.00 is reserved for any missing phenotype (Table 8.5). There is no fixed format for the number of values which can be seen in each line in Table 8.5. The user can arrange any number of phenotypic values in one line at their own preference.

Table 8.5 Phenotype information in a QTL mapping input file for MET

```
!****************************Phenotypic Data*****************************************
!-100.0 is resevred for missing phenotype
AuroraNY06b  92   92   88   87   100  89   90   -100 94   91   94   93   95   101  98   95   93
     88   107  98   -100 91   89   91   102  100  -100 89   93   -100 91   104  107  98   92   98
     108  90   91   -100 96   91   85   95   87   99   107  -100 104  90   106  104  101  86   90
     99   93   86   98   92   99   92   92   89   105  94   90   94   93   -100 92   93   94   97
     92   85   -100 93   98   94   99   98   106  97   99   107  88   93   100  103  88   92   93
     86   92   89   105  90   87   104  95   89   102  105  97   88   -100 94   89   -100 99   101
     91   88   93   95   91   104  91   98   91   94   91   107  107  91   107  104  90   96   94
     97   100  94   91   97   86   87   92   -100 90   89   97   95   90   94   89.5 -100 90   87
     98   95   91   91   88   103  98   94   94   98   91   94   86   92   98   95   106  101  106
     94   97   99   100  86   94   100  -100 105  90   92   96   94   88   87   92   88   107  88
     86   95   -100 99   91   94
ClaytonNC07b 73   71.3169   73   67.7337   82   69.4738   69   72   72   69.4235   71   68.371
     71.9926   72.1963   71   70.9856   71   72.3249   71.3249   69.6977   71   67   71.8485   72
     71   74.2184   73   70.7681   71   73   70   75   73.6549   73   73   73.6897   67   66.0813
     70.371   70   70   71   70.1097   77.2839   70   73.3567   73.3249   69   72.4235   70
     69.541   69.9915   68   67.9687   69   68   74.9943   72   69.7546   68.7774   73.4235   72
     67.5476   69.0993   67.9874   71.0928   71.5906   71   70   72   72   69   71.2184   71.6122
     70   67   73.541   73.0546   69.9889   70   71.8211   79.7432   69.4643   68.2202   70   73
     73.5273   74.9687   73   68.9227   67.7957   68.2733   71.5308   66.0712   70   71.2493   72
```

70 70 73.3169 69.541 68 75 70.1719 74.4738 71 72 74.4649 73 70.3124
70.4072 72 71 69.7624 68 74.5308 67.049 68 74 74 70 74 72.0478
74.2493 72 65 69.8485 72 71.4235 72.696 70.8433 74 72 71 73.3567
75.1913 69.6291 68 70 69 68.7352 71.476 72.371 68 70.2202 69 72.6977
72.3635 69.995 73 73.0928 74.3184 72.6549 70.312 71.2846 74 74.0419 70
65.1097 70 73.3562 70 72.9935 72.6529 73 75.0624 74 70.415 68.651 72
73.5702 76 71.7681 68.1675 70.4649 74 75.7968 72.4017 72 72 73 75.2394
69 69 77.4994 72.7432 67 73 70 71.4072 76.2846 71.7352 68.6038 71
ColumbiaMO06b 67 70 67 70 81 66 66 73 79 63 71 69 74 69 72 74 67
68 71 73 69 74 63 62 -100 -100 71 -100 67 71 71 63 66 77 78 72
65 -100 68 71 72 76 70 78 69 73 70 62 66 73 70 67 70 70 66
-100 75 67 65 72 74 75 61 73 72 67 66 66 -100 -100 67 70 66 73
61 62 71 66 67 67 78 78 72 66 67 67 68 76 -100 67 66 70 75
80 73 66 63 62 -100 -100 71 64 79 66 65 65 68 -100 74 63 65 68
66 75 73 72 66 74 76 70 75 69 73 74 67 63 72 76 67 79 72
72 -100 74 70 66 73 66 68 72 65 70 78 69 63 74 62 65 69 70
67 -100 74 69 66 75 75 65 66 70 75 74 71 -100 75 72 66 67 66
73 66 66 68 73 64 78 71 74 65 67 70 75 71 66 78 66 -100 70
65 66 68 65 67 65
ColumbiaMO07b 69 -100 -100 70 75 -100 -100 -100 -100 -100 72 -100 -100 -100 -100 -100
-100 -100 -100 -100 -100 -100 -100 -100 -100 -100 -100 72 -100 69 69 -100 -100 71
-100 -100 -100 -100 68 -100 66 -100 -100 -100 -100 -100 -100 -100 71 -100 -100 71 66
-100 69 -100 76 66 -100 67 73 -100 -100 -100 66 67 -100 68 -100 65 -100
-100 66 71 -100 63 -100 72 -100 -100 -100 -100 -100 -100 -100 71 -100 -100 -100
-100 -100 -100 -100 -100 -100 -100 -100 -100 -100 -100 64 -100 -100 -100 -100 -100 -100 70
-100 -100 -100 -100 -100 69 68 -100 69 -100 -100 68 71 -100 -100 -100 -100 64
-100 71 -100 -100 -100 -100 -100 69 71 -100 -100 -100 -100 -100 67 67 -100 -100
-100 67 -100 -100 66 65 -100 70 -100 -100 -100 -100 70 -100 -100 -100 -100 67
-100 -100 -100 -100 -100 -100 -100 -100 -100 -100 -100 -100 -100 -100 -100 67 -100 -100
-100 69 -100 64 -100 -100 -100 -100 63 -100 -100 -100 -100 67
Raleigh06b 72 72 71 70 78 70 71 74 74 69 74 71 72 74 71 74 73
74 71 70 74 66 74 72 70 74 74 73 75 73 70 73 74 73 72 70
68 68 70 70 74 70 73 76 72 76 75 67 70 71 70 72 66 70 68
67 76 70 70 72 75 73 66 70 69 70 70 72 70 72 73 74 75 70
72 72 73 72 70 69 75 78 74 72 70 75 71 75 70 70 67 69 70
70 74 70 75 73 70 75 71 74 74 71 71 71 71 76 74 72 70 71
68 70 70 72 70 72 75 77 74 74 74 75 70 67 71 71 70 74 70
73 70 76 72 72 70 69 74 76 72 77 74 69 73 69 70 72 68 69
74 70 73 72 72 72 75 67 68 73 75 73 73 68 72 76 70 70 66
70 74 74 72 69 72 75 76 74 70 70 73 75 69 68 74 74 70 67
72 72 74 66 70 74
UrbanaIL06b 81 76 77 78 86 75 77 77 81 75 84 81 75 78 75 84 79
80 79 -100 75 78 75 77 76 78 82 74 78 79 75 75 75 79 82 77
74 77 80 84 75 75 76 81 74 78 78 75 78 78 75 80 77 75 78

	75	81	75	77	78	82	76	72	82	75	76	75	80	76	74	75	78	81	77
	78	75	75	81	78	75	81	85	80	76	76	80	76	84	79	77	78	77	78
	73	80	75	76	80	76	74	73	75	80	75	76	75	81	84	78	74	78	80
	80	80	78	80	77	80	80	81	80	80	75	81	81	75	78	78	75	86	80
	79	79	81	79	82	76	79	75	80	76	81	80	77	80	79	77	78	75	75
	82	77	77	81	77	81	82	78	75	81	78	75	78	77	75	88	75	74	75
	81	80	82	76	77	78	75	84	78	78	81	86	81	78	73	86	75	75	76
	80	78	82	75	75	81													
UrbanaIL07b	73	78	69	70	74	73	70	68	71	69	70	69	70	71	73	73	73		
	70	73	70	73	69	70	-100	75	73	70	70	67	73	68	68	72	69	71	68
	70	67	70	70	70	70	70	69	67	70	72	68	69	73	68	70	73	68	70
	69	73	72	65	70	73	75	67	70	67	72	65	73	70	69	73	73	69	70
	65	68	73	73	69	69	73	75	70	67	73	73	70	75	73	69	70	68	69
	68	74	67	72	69	70	73	67	68	75	70	65	69	69	72	70	65	70	72
	69	73	70	70	70	73	73	73	73	73	70	70	73	67	70	68	73	78	69
	72	70	72	70	72	70	68	70	73	71	73	73	66	70	65	67	70	68	70
	71	66	73	70	70	73	73	67	70	72	73	69	70	65	73	73	70	70	65
	68	69	71	70	68	70	73	72	73	70	75	68	75	68	70	73	73	73	65
	70	70	75	70	67	73													

8.2 Input file for QTL by environment analysis in the EXCEL format

One mapping population for QTL mapping for multi-environmental trials can also be defined in an Excel file with the extension name ‘xls’ or ‘xlsx’. The file should be composed of five sheets: ‘GeneralInfo’ (similar to Table 8.1), ‘Chromosome’ (similar to Table 8.2), ‘LinkageMap’ (similar to Table 8.3), ‘Genotype’ (similar to Table 8.4), and ‘Phenotype’ (similar to Table 8.5).

8.3 Setting mapping parameters

The MET functionality can be initiated by (1) opening input files, or (2) double clicking MET files listed in the project window, or (3) clicking one MET file in the display window. When functionality MET is activated, the Display window shows the contents of the current input file, and the Parameter window is for interaction with users.

Additive mapping can be conducted for MET in IciMapping. The threshold LOD score declaring significant additive QTL can be specified.

8.3.1 Handling missing phenotype

The missing phenotypic data will be replaced by phenotypic mean of the trait.

8.3.2 Parameters for JICIM of QTL with additive effects

- Step (cM): the step in scanning represented by cM.
- Probability in stepwise regression: the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables (PIN). The largest P-value for removing variables is assumed to be two times of PIN.
- LOD Threshold: the threshold LOD score to declare significant QTL which is determined by manual input.

8.4 Outputs

In QTL IciMapping, the output files have the same prefex name as the input file but with different extension names. Four output files record some general information of the mapping population. Additive mapping has two kinds of output, denoted as R (for results at any scanning position) and Q (for significant QTL).

8.4.1 General information output files

1. STA file: Basic statistics of phenotypic data in the population (Table 8.6)

    - EnvironID: Environment ID represented by an integer number.
    - EnvironName: Environment name, same as given in the MET input file.
    - n: size of the mapping population.
    - Mean: Mean of the phenotypic data.
    - Variance: Variance of the phenotypic data.
    - Std: Standard deviation of the phenotypic data.
    - Skewness: Skewness of the phenotypic data.
    - Kurtosis: Kurtosis of the phenotypic data.
    - Min: Minimum value of the phenotypic data.
    - Max: Maximum value of the phenotypic data.
    - Range: Range of the phenotypic data.
    - W-test: The Shapiro Wilk W-statistic for the test of normality.
    - P-Value: P-value of the W-test of normality.

Table 8.6 Basic statistics of phenotypic data in the population (STA)

EnvironID	EnvironName	n	Mean	Variance	Std	Skewness	Kurtosis	Min	Max	Range	W-test	P-value
1	AuroraNY06b	180	94.7694	34.4060	5.8657	0.5486	-0.5700	85.0000	108.0000	23.0000	0.9259	0.0000
2	ClaytonNC07b	194	71.3173	6.3247	2.5149	0.4636	1.4184	65.0000	82.0000	17.0000	0.9795	0.3012
3	ColumbiaMO06b	179	69.6648	19.7634	4.4456	0.3015	-0.6302	61.0000	81.0000	20.0000	0.9535	0.0000
4	ColumbiaMO07b	51	68.4706	8.3741	2.8938	0.2330	-0.1465	63.0000	76.0000	13.0000	0.9696	0.3567
5	Raleigh06b	194	71.7577	6.5369	2.5567	-0.0607	-0.4620	66.0000	78.0000	12.0000	0.9541	0.0000
6	UrbanaIL06b	193	78.0725	9.0572	3.0095	0.6371	0.0994	72.0000	88.0000	16.0000	0.9348	0.0000
7	UrbanaIL07b	193	70.4456	6.4983	2.5492	0.0218	-0.0953	65.0000	78.0000	13.0000	0.9383	0.0000

2. COE file: Correlation coefficient matrix between markers (Table 8.7)

   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: Marker name, same as given in the MET input file.
   - First row: Number after MarkerID and MarkerName is also Marker ID.
   - Others: Correlation coefficient between two markers.

Table 8.7 Correlation coefficient matrix between markers (COE, incomplete)

MarkerID	MarkerName	1	2	3	4	5	6	……
1	L00411	1.0000	0.8977	0.6822	0.5896	0.5162	0.4655	……
2	L00569	0.8977	1.0000	0.7832	0.6488	0.5765	0.5246	……
3	L00068	0.6822	0.7832	1.0000	0.8030	0.7311	0.6785	……
4	L01003	0.5896	0.6488	0.8030	1.0000	0.9280	0.8339	……
5	L00196	0.5162	0.5765	0.7311	0.9280	1.0000	0.9073	……
6	L00609	0.4655	0.5246	0.6785	0.8339	0.9073	1.0000	……

……

3. MTP file: There are two parts in this output file. The first part contains information for each marker (Table 8.8), and the second part gives the marker types after imputation of missing markers (Table 8.9). Markers are in the order as in the linkage map.

   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: name of each marker
   - Chromosome ID: ID number starting from 1 for each chromosome
   - ChromosomeName : name of each chromosome
   - Position (cM): the position of the current marker in the linkage group
   - n(AA): the number of genotypes same as the first parent (P1), which denoted as 2 in the input file.
   - n(Aa): the number of genotypes same as F1, which denoted as 1 in the input file.
   - n(aa): the number of genotypes same as the second parent (P2), which denoted as 0 in the input file.
   - n(-): the number of missing genotypes, which denoted as -1 in the input file.
   - ChiSquare: χ^2-test statistics testing for segregation distortion of markers.
   - P-Value: the corresponding probability for the χ^2-test statistics, i.e., which is equal to P(x> ChiSquare).

Table 8.8 Marker information and test of segregation distortion (MTP Part 1, incomplete)

MarkerID	MarkerName	Chromosome	Position	n(AA)	n(Aa)	n(aa)	n(-)	Chi^2-Test	P(x>Chi^2)
1	L00411	1	0.0000	87	0	90	17	0.0508	0.8216
2	L00569	1	3.7000	87	0	94	13	0.2707	0.6029
3	L00068	1	9.7000	81	0	92	21	0.6994	0.4030
4	L01003	1	13.4000	0	0	0	194	NaN	NaN
5	L00196	1	15.6000	85	0	92	17	0.2768	0.5988
6	L00609	1	17.9000	0	0	0	194	NaN	NaN

……

Table 8.9 Marker types after imputation of missing markers (MTP Part 2, incomplete)

```
!*********************************Marker Type*****************************************
!Marker type: 2 for P1; 1 for F1; 0 for P2; BC1=F1xP1; BC2=F1xP2; -1 for missing markers
L00411              0  2  0  0  0  2  2  0  2  0  0  2  0  2  2  2  0  0  0
2  2  0  2  2  2  0  0  0  2  2  2  2  2  0  2  2  2  0  2  2  0  2  2  0
2  0  2  2  2  0  2  0  0  0  2  2  2  0  0  2  2  2  2  0  0  0  2  0  2
2  2  0  2  0  0  2  2  2  2  0  0  0  2  2  2  2  0  2  2  2  2  0  0  0
2  0  2  0  2  0  2  0  0  2  2  0  0  0  2  2  2  0  2  2  0  0  0  2  0
0  2  0  2  0  2  2  0  0  2  0  0  2  0  0  0  2  0  2  0  0  0  2  2  0
2  0  0  0  2  0  0  2  2  0  0  2  0  2  0  0  2  2  2  0  2  0  0  2  2
2  0  0  0  0  2  0  0  2  2  0  0  2  0  0  0  2  0  2  2  0  0  0  2  0
L00569              0  2  0  0  0  2  2  0  2  0  0  2  0  2  2  2  0  0  0
0  2  0  2  0  2  2  0  0  2  2  2  2  2  0  2  2  2  0  2  2  0  2  2  0
2  0  2  2  2  0  2  0  0  0  2  2  2  0  0  2  2  2  2  0  0  0  2  0  2
2  2  0  2  0  0  2  2  2  2  0  0  0  2  2  2  2  0  2  2  0  2  0  0  0
0  0  2  2  2  0  2  0  0  2  2  0  0  0  2  2  2  0  2  2  0  0  0  2  0
0  2  0  2  0  2  2  0  0  2  0  0  2  0  0  0  2  0  0  0  0  0  2  2  0
2  0  0  0  2  0  0  2  2  0  0  2  0  2  0  0  2  2  2  0  2  0  0  2  2
2  0  0  0  0  0  0  0  2  2  0  0  2  0  0  0  2  2  2  2  0  0  0  0  0
L00068              0  2  0  0  0  2  2  0  2  0  0  2  0  2  2  2  0  0  0
0  2  0  2  0  0  2  0  0  2  2  2  2  2  0  2  2  0  0  2  2  0  2  2  0
2  0  2  2  2  0  2  0  0  0  2  0  2  2  0  2  0  2  2  0  0  0  2  0  2
2  2  2  2  0  0  2  2  2  2  2  0  0  0  0  2  0  2  2  2  0  2  0  0  0
0  0  0  2  2  0  2  0  0  2  2  0  2  0  2  2  2  0  2  2  0  0  0  2  2
0  2  0  2  0  2  2  0  0  2  0  0  2  0  0  0  2  2  0  0  0  0  0  2  0
2  0  0  0  2  2  0  2  2  0  0  2  0  0  0  0  2  2  2  0  2  0  0  2  2
2  0  0  0  0  0  0  0  2  2  0  0  0  0  2  0  0  2  2  2  0  0  0  0  0
L01003              0  2  0  0  0  2  0  0  2  0  0  2  0  0  2  0  2  2  2  0  0  0
0  2  0  2  2  2  2  0  2  2  2  2  2  0  2  2  0  0  2  0  0  2  2  2  0
0  0  0  2  2  0  2  0  0  0  2  0  2  2  0  2  2  2  2  0  0  0  2  0  2
2  0  2  2  0  0  0  2  2  2  2  0  0  0  0  2  0  2  2  2  0  0  0  0  0
2  0  0  2  2  0  2  0  0  2  2  0  2  0  2  2  2  0  2  2  0  0  0  2  2
0  2  0  2  0  2  2  0  0  2  0  0  2  0  2  0  0  2  0  0  0  0  0  2  0
2  0  0  0  2  2  2  2  2  0  0  2  0  0  0  0  2  2  2  0  2  0  0  2  2
2  0  0  0  0  0  0  0  0  2  0  0  2  0  2  0  0  2  0  2  0  2  0  0  0
L00196              0  2  2  0  0  2  0  0  2  2  0  0  2  0  0  2  0  2  2  2  0  0  0
0  2  0  2  2  2  2  0  2  2  2  2  2  0  2  2  0  0  2  0  0  2  2  2  0
0  0  0  2  2  0  2  0  0  0  2  0  2  2  0  2  2  2  2  0  0  0  2  2  2
2  0  2  2  0  0  0  2  2  2  2  0  0  0  0  2  0  2  2  2  0  0  0  0  0
2  2  0  2  2  0  2  2  0  2  2  0  2  0  2  2  2  0  2  2  0  0  0  2  2
0  2  0  2  0  2  2  0  0  2  0  0  2  0  2  2  0  2  0  0  0  0  0  2  0
2  0  0  0  2  2  2  2  2  0  0  2  0  0  0  0  2  2  2  0  2  0  0  0  0
2  0  0  0  0  0  0  0  2  0  0  2  0  2  0  0  2  0  2  0  2  0  0  0  0
L00609              0  2  2  0  0  2  0  0  2  0  0  2  0  2  0  0  2  0  2  2  0  2  0
0  2  0  2  2  2  2  0  0  2  2  2  2  2  0  2  0  0  0  2  2  0  2  2  0
0  0  0  2  2  0  2  0  0  0  2  0  2  2  0  2  2  2  0  0  0  0  2  2  2
2  0  2  2  0  0  0  2  2  2  2  0  0  0  0  2  0  2  2  2  0  0  0  0  0
2  2  0  2  2  0  2  2  0  2  2  0  2  0  2  2  2  0  2  2  0  0  0  0  2
0  2  0  0  0  2  2  2  0  2  0  0  2  0  2  2  0  2  0  0  0  0  0  2  0
2  0  0  0  2  2  2  2  2  0  0  2  0  0  0  0  2  2  2  0  2  0  0  0  0
2  0  0  0  0  0  0  0  0  2  0  0  2  0  2  0  0  2  0  0  0  2  0  0  0
……
```

4. STP file: Results from stepwise regression (Table 8.10)

- EnvironID: Environment ID represented by an integer number.
- EnvironName: Environment name, same as given in the MET input file.
- Type: To distinct between the two stepwise regressions for additive and epsiatsis mapping.
- Para: Parameter coefficient after the stepwise regression.
- Numbers after Para are markers retained in the stepwise regression.
- Intercept: Intercept of the stepwise regression.
- R^2: Phenotypic variation explained by the final regression model. In QTL additive mapping, this can be viewed as the total phenotypic variation explained by all additive QTL. In QTL espistasis mapping, this can be viewed as the total

phenotypic variation explained by all QTL interactions.

Table 8.10 Retained markers and their coefficients in stepwise regression (STP)

EnvironID	EnvironName	Type	Para	35	Intercept	R^2
1	AuroraNY06b	ADD	COEF	-1.5064	95.1422	6.2249

……

8.4.2 Results from all scanning markers or chromsomal positions

RAD file: Results from additive mapping for MET at any tesing positions (Table 8.11)

- Chromosome: Chromosome ID represented by an integer number.
- Position: The scanning position in cM on the chromosome.
- LODa: LOD score for additive and dominance effects.
- LODae: LOD score for additive and dominance by environment effects.
- Va: Phenotypic variation expelained by additive and dominance effect at the current scanning position.
- Vae: Phenotypic variation expelained by additive and dominance by environment effect at the current scanning position.
- LOD_01, LOD_02,⋯: LOD score of additive and dominance effects at the current scanning position for each environment.
- ADD_01, ADD_02,⋯: Estimated additive effect of QTL at the current scanning position for each environment.
- DOM_01, DOM_02,⋯: Estimated dominance effect of QTL at the current scanning position for each environment.

Table 8.11 Results of additive mapping (RAD, incomplete)

Chromosome	Position	LODa	LODae	Va	Vae	LOD_01	LOD_02	LOD_03	LOD_04	LOD_05	LOD_06	LOD_07	ADD_01	ADD_02	ADD_03	ADD_04	ADD_05	ADD_06	ADD_07
1	0.0000	1.3324	2.5157	0.0452	0.0613	0.5775	0.0876	0.3202	0.0409	0.0980	0.1442	0.0715	0.0949	-0.2767	-0.5383	-0.0752	-0.285	-0.5198	0.1123
1	1.0000	1.1661	2.6688	0.0393	0.0654	0.4569	0.0902	0.3036	0.0362	0.0928	0.1341	0.0558	0.1624	-0.2694	-0.5150	-0.075	-0.2788	-0.5228	0.1101
1	2.0000	1.0059	2.7845	0.0339	0.0709	0.3772	0.0820	0.2693	0.0314	0.0823	0.1187	0.0463	0.2308	-0.2617	-0.4925	-0.0751	-0.2726	-0.5269	0.1084
1	3.0000	0.8597	2.8566	0.0290	0.0778	0.3354	0.0657	0.2225	0.0271	0.0679	0.0986	0.0416	0.2999	-0.2537	-0.4711	-0.0752	-0.2664	-0.5321	0.1071

……

8.4.3 Results files for significant QTL

QAD file: Significant QTL from additive mapping for MET (significant additive and dominance QTL can be selected from this file; Table 8.12).

- Chromosome: Chromosome ID represented by an integer number.
- Position: The scanning position in cM on the chromosome.
- LeftMarker: Name of the left-side marker of the identified QTL.
- RightMarker: Name of the right-side marker of the identified QTL.
- LODa: LOD score for additive and dominance effects.

- LODae: LOD score for additive and dominance by environment effects.
- Va: Phenotypic variation expelained by additive and dominance effect at the current scanning position.
- Vae: Phenotypic variation expelained by additive and dominance by environment effect at the current scanning position.
- LOD_01, LOD_02,⋯: LOD score of additive and dominance effects at the current scanning position for each environment.
- ADD_01, ADD_02,⋯: Estimated additive effect of QTL at the current scanning position for each environment.
- DOM_01, DOM_02,⋯: Estimated dominance effect of QTL at the current scanning position for each environment.

Table 8.12 Significant QTL for additive mapping (QAD, incomplete)

Chromosome	Position	LeftMarker	RightMarker	LODa	LODae	Va	Vae	LOD_01	LOD_02	LOD_03	LOD_04	LOD_05	LOD_06	LOD_07	ADD_01	ADD_02	ADD_03	ADD_04	ADD_05	ADD_06	ADD_07
1	10.0000	L00068	L01003	3.1337	2.7163	0.1037	0.0500	1.3233	0.2083	0.7413	0.1083	0.2410	0.3558	0.1484	-0.5568	-0.2156	-0.3629	-0.2346	-0.3977	-0.6014	0.1145
1	83.0000	L00388	L00789	8.5059	8.4357	0.2877	0.1099	3.6667	0.5201	2.0328	0.2568	0.6037	0.8901	0.5307	0.4243	0.1231	1.1257	0.2953	0.4562	0.3999	0.9305
1	124.0000	L00780	L00795	4.1006	0.8787	0.1463	0.2249	1.8281	0.2585	0.9656	0.1203	0.2895	0.4362	0.2062	-1.4815	-0.3783	-0.4765	-0.0706	-0.1109	-0.1233	-0.0366
……																					

Chapter 9. QTL Mapping for the NAM Design (NAM)

9.1 Input file for the NAM design (*.nam)

9.1.1 General information of the mapping population

Six parameters were used for the general information defining a NAM design mapping population (Table 9.1).

- Mapping Function: specify the mapping function which will be used to transfer recombination frequency to mapping distance, or from mapping distance to recombination frequency.
  - 1 for Kosombi mapping function.
  - 2 for Haldane mapping function.
  - 3 for Morgan mapping function.

- Marker Space Type: specify whether the markers on a chromosome (or linkage group) are defined by positions or marker intervals.
  - 1 for intervals, i.e. the number behind a marker is the distance of the marker to its next marker. 0 is normally given for the last marker on a chromosome or a linkage group.
  - 2 for positions, i.e. the number behind each marker is the position of the marker on the chromosome or the linkage group.

- Marker Space Unit: specify the unit used in marker linkage group.
  - 1 for centi-Morgan (cM).
  - 2 for Morgan (M). 1 M = 100 cM.

- Number of Chromosomes: specify the number of chromosomes (or linkage groups) in the mapping population.

- Number of Traits: number of traits phenotyped in the mapping population.

- Number of Families: number of families contained in the NAM design mapping population.

Table 9.1 General population information in NAM design mapping input file.

```
!*************** Note: lines staring with "!" are remark and will be ignored in the program**************
!****************************** General Information ***************************************************
 2         !Mapping Function (1 for Kosambi Function; 2 for Haldane Function; 3 for Morgan mapping function)
 2         !Marker Space Type (1 for intervals; 2 for positions)
 1         !Marker Space Unit(1 for centiMorgans; 2 for Morgan)
 5         !Number of Chromosomes (or Linkage Group)
 1          !Number of traits
 3          !Number of families
```

9.1.2 Family information

Three parameters were used for the family information defining a NAM design mapping population (Table 9.2).

- Family name.
- The number of individuals in each family.
- Population Type for each family: describe the type of the population. Assuming F1 = P1 x P2, the 4 biparental populations are:
  - 1. P1BC1F1: the backcross population where the first parent (P1) is used as the recurrent.
  - 2. P2BC1F1: the backcross population where the second parent (P2) is used as the recurrent.
  - 3. F1DH: doubled haploids derived from F1.
  - 4. RIL: recombination inbred lines derived from repeated selfing since F1 generation.

Table 9.2 Family information in NAM design mapping input file

```
!********* ******** Family information ***********************************
!Family name    Numbers of individuals in each population
An   120 4
Kas  164 4
Kond  121 4
```

9.1.3 Linkage group or chromosome information

The name of each chromosome and the number of markers on the chromosome were specified first (Table 9.3), followed by the definition of each chromosome (Table 9.4). Each chromosome was defined by all markers on it and all marker positions.

Table 9.3 Linkage group information in NAM design mapping input file

```
!********************** Information for Chromosomes and Markers *****************
!Chromosome    NumMarkers in each chromosome
-Ch1 25
-Ch2 19
-Ch3 19
-Ch4 22
-Ch5 23
```

Table 9.4 Definition of each chromosome (incomplete)

```
!MarkerName; Chromosome; position or interval as indicated by General Information-Ch1
nga59    1   0
SNP5     1   3.4
SNP388   1   8.3
SNP107   1   11.4
F3F19    1   16.3
SNP132   1   22.6
SNP251   1   27.4
SNP100   1   32.5
SNP65    1   36.1
SNP32    1   37.2
SNP373   1   45
SNP158   1   49.9
SNP279   1   54.8
T27K12   1   55.6
CIW1     1   65.2
```

```
F6D8.94  1   69.4
nga128   1   73.8
```

……

9.1.4 Marker type information

Marker types are arranged by the ordered markers defined in Table 9.4. That is, the marker types for all individuals on the first marker were given first, then followed by the second marker, and so on (Table 9.5). The marker name in this part has to be the same as that specified in Table 9.4. In IciMapping, 2 was used to represent the marker type of the first parent (P1), 0 for the second parent (P2), 1 for the F1 marker type, and -1 for any missing markers. Any missing markers will be assigned values based on the types of their neighboring markers. The number of marker types behind each marker name has to be exact the population size.

Table 9.5 Marker type information in NAM design mapping input file (incomplete)

```
!******************************* Marker Types **************************************
!Marker type: 0 for common parent (CP); 2 for the other parent (OP); -1 for missing markers
nga59   0   0   2   2   0   2   0   0   0   0   0   2   0   2   0   0   2   0
    0   0   2   2   0   0   0   2   0   0   2   2   0   0   0   0   0   0   2
    0   0   2   2   0   0   0   0   0   2   2   2   2   2   0   2   2   -1  2
    2   2   2   0   2   0   0   0   0   0   2   2   0   2   0   2   2   0   2
    0   2   2   0   2   2   2   0   2   0   0   0   0   0   0   0   2   0   2
    2   -1  2   0   2   -1  -1  2   0   2   0   0   2   2   0   2   0   0   0
    0   2   0   0   2   2   0   2   0   0   0   0   2   2   0   0   2   2   0
    2   0   0   2   2   0   0   0   2   0   -1  0   2   0   2   0   2   2   0
    0   0   2   2   -1  2   0   0   -1  2   0   2   0   0   2   -1  0   0   -1
    0   0   0   0   0   -1  0   0   2   2   0   0   -1  0   2   0   0   0   2
    0   -1  2   2   0   2   0   0   0   2   0   2   2   0   2   2   2   0   0
    0   0   2   2   0   0   0   2   2   0   2   0   2   0   0   0   0   2   0
    2   0   0   0   0   -1  -1  0   2   0   0   2   2   0   2   2   2   0   2
    0   2   0   2   -1  2   0   2   2   2   2   2   0   -1  2   0   2   0   0
    0   2   0   0   0   2   2   2   0   0   2   0   2   0   0   0   0   2   -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1
SNP5-1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
    -1  -1  -1  -1  -1  -1  2   0   0   0   -1  2   2   0   0   2   2   -1  2
    0   0   2   0   2   0   0   2   0   -1  0   0   0   2   0   2   -1  0   0
    0   2   2   -1  2   0   0   -1  2   2   0   0   0   2   2   0   0   -1  0
    0   0   0   0   -1  0   0   2   2   0   0   -1  0   2   2   0   0   2   0
    -1  2   2   0   -1  0   0   0   2   2   2   2   0   2   2   2   0   0   -1
    0   2   2   -1  0   0   2   2   0   2   2   2   0   0   0   0   2   0   2
    0   0   0   0   -1  0   0   2   0   0   2   2   0   2   2   2   0   2   0
    2   0   2   0   2   0   2   2   2   2   2   2   0   2   0   2   0   0   0
    2   0   0   0   2   2   2   0   0   2   0   2   0   0   2   0   2   -1  0
    -1  0   2   2   -1  2   0   0   0   2   2   2   2   0   2   2   0   0   0
    -1  2   2   0   2   2   2   2   0   0   2   0   0   2   2   2   0   0   2
    2   2   0   2   0   2   0   2   0   0   0   0   0   0   0   0   0   0   2
    0   0   0   2   0   0   2   2   2   0   -1  2   0   0   0   2   2   2   2
    2   0   2   2   0   2   0   0   2   0   2   2   0   2   2   2   0   0   2
    0   -1  2   0   0   0   0   2   0   0   0   0   2   2   2   2   0   0   2
    2   0   2   2   2   2
```

……

9.1.5 Phenotype information

Phenotypes were first given for the first trait, then for the second trait and so on. -100.00 is reserved for any missing phenotype (Table 9.6). There is no fixed format for the number of values which can be seen in each line in Table 9.6. The user can arrange any number of phenotypic values in one line at their own preference.

Table 9.6 Phenotypic information in NAM design mapping input file

```
!***************************** Phenotypic Data *********************************
!-100.0 is resevred for missing phneotype
Floweringtime    26.0 21.8 21.8 23.0 23.5 22.3 22.5 21.9 23.5 28.3 22.9 26.5 22.6 22.9 23.8
    22.3 22.1 23.9 23.0 22.4 23.6 22.8 23.4 22.1 20.5 22.0 26.3 25.9 22.3 26.6 25.1 23.1 24.3
    26.3 22.0 23.1 23.5 22.0 22.6 21.9 21.9 22.5 22.3 21.5 26.0 21.9 23.6 23.6 22.5 22.0 25.3
    22.1 23.4 22.1 23.0 26.1 19.1 21.5 21.5 23.5 20.8 23.5 22.9 20.8 26.0 27.0 23.0 23.4 25.4
    21.8 25.4 22.5 23.0 23.3 25.4 23.6 22.9 21.5 24.0 22.5 22.5 28.1 22.6 25.0 22.9 22.4 22.4
    22.1 23.3 23.1 22.1 20.8 23.5 21.9 21.1 23.1 22.1 24.5 21.9 22.3 26.1 24.1 22.8 22.9 24.9
    25.4 23.5 22.3 23.6 22.5 21.5 22.5 21.5 21.6 22.5 23.3 23.3 23.6 21.6 24.5 26.8 43.2 34.4
    35.5 26.5 33.7 31.7 35.9 29.5 36.2 27.7 25.7 34.4 33.7 44.2 36.3 31.2 42.0 35.7 40.5 37.1
    34.8 38.2 34.5 31.1 31.5 32.3 28.3 29.2 31.5 28.3 28.4 36.2 31.7 50.5 35.8 31.3 43.9 35.9
    35.7 32.4 33.2 37.6 36.8 34.9 38.2 34.3 51.5 47.2 37.2 30.3 45.3 33.6 28.3 35.8 31.7 27.2
    34.1 29.3 30.8 37.7 35.2 35.8 26.1 30.8 28.8 34.9 33.7 31.8 32.9 31.4 32.5 28.2 41.9 36.7
    29.5 31.5 28.6 32.6 33.4 33.4 33.2 30.1 41.9 39.9 37.5 39.7 50.9 38.9 25.4 39.6 37.0 35.5
    42.3 34.6 28.9 38.0 32.9 33.5 48.9 41.0 31.1 34.6 40.6 36.3 39.5 39.4 33.2 28.3 32.6 40.6
    36.6 31.6 30.6 40.4 34.0 25.5 34.0 26.2 46.3 46.6 53.2 24.9 30.9 36.8 33.2 36.9 26.4 32.5
    31.0 34.9 35.0 46.6 31.2 26.9 29.2 33.0 27.8 35.3 26.1 31.9 29.9 29.6 28.5 47.7 31.8 30.9
    35.9 32.5 32.9 32.4 30.2 33.3 33.9 32.3 38.6 54.0 44.5 34.8 29.5 32.1 35.7 38.7 31.6 35.7
    32.3 30.3 38.0 32.5 40.2 37.0 50.6 27.2 32.3 48.5 33.7 35.5 33.3 27.3 38.5 53.3 49.0 45.8
    44.5 28.2 35.3 30.2 33.8 36.2 26.8 33.0 31.0 29.0 33.3 37.0 31.2 35.7 38.0 66.2 32.3 35.8
    45.5 29.0 48.3 46.0 30.2 38.7 44.5 45.2 41.0 49.7 49.2 48.0 38.7 37.7 37.5 33.3 43.7 28.8
    42.0 33.8 33.8 32.2 35.5 48.0 42.5 51.7 28.2 41.7 42.7 31.5 40.2 42.0 26.7 36.7 43.2 46.2
    42.7 32.2 30.7 46.3 39.2 38.7 31.2 28.8 36.5 29.0 41.5 35.5 51.3 38.3 52.3 46.8 49.5 34.0
    47.0 29.2 31.3 33.5 50.7 57.3 43.2 27.7 29.0 35.2 41.7 31.2 28.2 34.8 32.7 45.3 41.2 40.8
    35.8 53.0 30.3 52.5 27.3 45.7 50.2 39.8 30.0 38.5 52.7 39.2
```

9.2 Input file for the NAM design in the EXCEL format

One mapping population for QTL mapping in NAM design populations can also be defined in an Excel file with the extension name ‘xlsx’. The file should be composed of six sheets: ‘GeneralInfo’ (similar to Table 9.1), ‘Family’ (similar to Table 9.2, ‘Chromosome’ (similar to Table 9.3), ‘LinkageMap’ (similar to Table 9.4), ‘Genotype’ (similar to Table 9.5), and ‘Phenotype’ (similar to Table 9.6).

9.3 Setting mapping parameters

The NAM functionality can be initiated by (1) opening input files, or (2) double clicking NAM files listed in the project window, or (3) clicking one NAM file in the display window. When functionality NAM is activated, the Display window shows the contents of the current input file, and the Parameter window is for interaction with users.

Additive mapping can be conducted for NAM in IciMapping. The threshold LOD score declaring significant additive QTL can be specified.

9.3.1 Handling missing phenotype

The missing phenotypic data will be replaced by phenotypic mean of the trait.

9.3.2 Parameters for JICIM of QTL with additive effects

- Step (cM): the step in scanning represented by cM.
- Probability in stepwise regression: the largest P-value for entering variables in stepwise regression of residual phenotype on marker variables (PIN). The largest P-value for removing variables is assumed to be two folds of PIN.
- LOD Threshold: the threshold LOD score determined by the users to declare the significant QTL.

9.4 Outputs

In QTL IciMapping, the output files have the same prefix name as the input file but with different extension names.

9.4.1 General information output files

1. MTP file: There are two parts in this output file. The first part contains information for each marker (Table 9.7), and the second part gives the marker types after imputation of missing markers (Table 9.8). Markers are in the order as in the linkage map.

    - Family: Family ID represented by an integer number.
    - MarkerID: Marker ID represented by an integer number.
    - MarkerName: name of each marker.
    - Chromosome: ID number starting from 1 for each chromosome.
    - Position (cM): the position of the current marker in the linkage group.
    - n(AA): the number of genotypes in each family same as the first parent (P1), which denoted as 2 in the input file.
    - n(Aa): the number of genotypes in each family same as F1, which denoted as 1 in the input file.
    - n(aa): the number of genotypes in each family same as the second parent (P2), which denoted as 0 in the input file.
    - n(-): the number of missing genotypes in each family, which denoted as -1 in the input file.
    - Chi^2-Test: χ^2-test statistics testing for segregation distortion of markers.
    - P(x>Chi^2): the corresponding probability for the χ^2-test statistics, i.e., which is equal to P(x>Chi^2-Test).

Table 9.7 Marker information and test of segregation distortion (MTP Part 1, incomplete)

Family	MarkerID	MarkerName	Chromosome	Position	n(AA)	n(Aa)	n(aa)	n(-)	Chi^2-Test	P(x>Chi^2)
1	1	nga59	1	0.0000	30	0	39	51	1.1739	0.2786
1	2	SNP5	1	3.4000	27	0	40	53	2.5224	0.1122
1	3	SNP388	1	8.3000	30	0	36	54	0.5455	0.4602
1	4	SNP107	1	11.4000	27	0	41	52	2.8824	0.0896
1	5	F3F19	1	16.3000	25	0	40	55	3.4615	0.0628
1	6	SNP132	1	22.6000	22	0	44	54	7.3333	0.0068
1	7	SNP251	1	27.4000	31	0	37	52	0.5294	0.4669
1	8	SNP100	1	32.5000	24	0	43	53	5.3881	0.0203
1	9	SNP65	1	36.1000	32	0	37	51	0.3623	0.5472
1	10	SNP32	1	37.2000	33	0	34	53	0.0149	0.9028

……

Table 9.8 Marker types after imputation of missing markers (MTP Part 2, incomplete)

```
!*************************************** Marker Types ***********************************************
!Marker type: 0 for common parent (CP); 2 for the other parent (OP); -1 for missing markers
nga59                0  0  2  2  0  2  0  0  0  0  0  2  0  2  0  0  2  0  0  0  2  2  0  0  0
2  0  0  2  2  0  0  0  0  0  0  2  0  0  2  2  0  0  0  0  0  2  2  2  2  2  0  2  2  0  2
2  2  2  0  2  0  0  0  0  0  2  2  0  2  0  2  2  0  2  0  2  2  0  2  2  2  0  2  0  0  0
0  0  0  0  2  0  2  2  2  0  2  2  0  2  0  0  2  0  0  2  2  0  2  0  0  0  0  2  0  0  2
2  0  2  0  0  0  0  2  2  0  0  2  2  0  2  0  0  2  2  0  0  0  2  0  2  0  2  0  2  0  2
2  0  0  0  2  2  2  2  0  0  0  2  0  2  0  0  2  2  0  0  0  0  0  0  0  0  0  0  0  2  2
0  0  2  0  2  0  0  0  2  0  2  2  2  0  2  0  0  0  2  0  2  2  2  2  2  0  0  0  0  0  2
2  0  0  0  2  2  0  2  0  2  0  0  0  0  2  0  2  0  0  0  0  2  0  0  2  2  0  2  2  0  2
2  2  0  2  0  2  0  2  0  2  0  2  2  2  2  2  2  0  0  2  0  2  0  0  0  2  0  0  0  2  2  2
0  0  2  0  2  0  0  0  0  2  2  2  0  0  2  2  2  2  0  0  0  2  2  2  2  0  2  2  2  0  0
0  0  2  0  2  2  2  2  0  0  2  0  0  2  2  2  0  0  0  2  2  0  2  0  2  0  2  0  0  0  0
0  0  0  0  0  0  2  0  0  0  2  0  0  2  2  0  2  0  2  0  0  0  2  2  2  2  2  2  2  2  0
2  0  0  2  0  2  2  0  2  2  2  0  0  2  0  0  2  0  0  0  0  2  0  0  0  0  2  2  2  2  0
0  2  2  0  2  2  2  2
SNP5                 2  0  2  2  0  2  0  0  0  0  0  2  0  2  0  0  2  0  0  0  2  2  0  0  0
2  0  0  2  2  0  0  0  0  0  0  2  0  0  2  2  0  0  0  0  0  2  2  2  2  2  0  2  2  0  2
2  2  2  0  2  0  0  0  0  0  2  2  0  2  0  2  2  0  2  0  2  2  0  2  2  2  0  2  0  0  0
0  0  0  0  2  0  2  2  2  2  0  2  2  0  2  0  0  2  0  0  2  2  0  2  0  0  0  0  2  0  2
2  0  2  0  0  0  0  2  2  0  0  2  2  0  2  0  0  2  0  2  0  0  2  0  2  0  0  0  2  0  2
2  0  0  0  2  2  2  2  0  0  0  2  2  0  0  0  2  2  0  0  0  0  0  0  0  0  0  0  0  2  2
0  0  2  0  2  2  0  0  2  0  2  2  0  2  0  0  0  0  2  2  2  0  2  2  0  0  0  0  0  0  2
2  0  0  0  2  2  0  2  2  2  0  0  0  0  2  0  2  0  0  0  0  2  0  0  2  0  0  2  2  0  2
2  2  0  2  0  2  0  2  0  2  0  2  2  2  2  2  2  0  2  0  2  0  0  0  2  0  0  0  2  2  2
0  0  2  0  2  0  0  2  0  2  2  2  0  0  0  0  2  2  2  0  2  2  2  0  2  2  0  2  0  0  0
0  2  2  0  2  2  2  2  0  0  2  0  0  2  2  2  0  0  2  2  2  0  2  0  2  0  2  0  0  0  0
0  0  0  0  0  0  2  0  0  0  2  0  0  2  2  2  0  0  2  0  0  0  2  2  2  2  2  0  2  2  0
2  0  0  2  0  2  2  0  2  2  2  0  0  2  0  0  2  0  0  0  0  2  0  0  0  0  2  2  2  2  0
0  2  2  0  2  2  2  2
……
```

2. STP file: Results from stepwise regression (Table 9.9)

- TraitID: Trait ID represented by an integer number.
- TraitName: Trait name, same as given in the NAM input file.
- Type: To distinct between the two stepwise regressions for additive and epistasis mapping.
- Para: Parameter coefficient after the stepwise regression.
- Numbers after Para are markers retained in the stepwise regression.
- Intercept: Intercept of the stepwise regression.
- R^2: Phenotypic variation explained by the final regression model. In QTL additive mapping, this can be viewed as the total phenotypic variation explained by all additive QTL. In QTL espistasis mapping, this can be viewed as the total phenotypic variation explained by all QTL interactions.

Table 9.9 Retained markers and their coefficients in stepwise regression (STP)

TraitID	TraitName	Type	Para	85	181	……	434	Intercept	R^2
1	Floweringtime	ADD	COEF	1.6546	1.8522	……	-7.9649	30.2163	83.0239

9.4.2 Results from chromosomal positions

RAD file: Mapping results from JICIM for QTL with additive effects at any testing

positions (Table 9.10)

- Chromosome: Chromosome ID represented by an integer number.
- Position: Marker position in cM on the chromosome.
- LOD: joint LOD score at the current scanning position calculated from JICIM.
- *_LOD: LOD score at the current scanning position in each family.
- *_ADD: Additive effect at the current scanning position in each family.

Table 9.10 Results of JICIM (RAD, incomplete)

Chromosome	Position	LOD	An_LOD	An_ADD	Kas_LOD	Kas_ADD	Kond_LOD	Kond_ADD
1	0.0000	0.4402	0.0071	0.0594	0.4114	-0.8309	0.0220	0.2550
1	1.0000	0.3894	0.0071	0.0597	0.3689	-0.7891	0.0135	0.1996
1	2.0000	0.3353	0.0071	-0.0597	0.3212	-0.7310	0.0069	0.1421
1	3.0000	0.2785	0.0058	-0.0537	0.2706	-0.6559	0.0024	0.0837
1	4.0000	0.2873	0.0234	-0.1002	0.2557	-0.6896	0.0084	0.1596
1	5.0000	0.3007	0.0347	-0.1221	0.2559	-0.6930	0.0104	0.1768
1	6.0000	0.3037	0.0481	-0.1434	0.2432	-0.6777	0.0124	0.1930
1	7.0000	0.2802	0.0634	-0.1637	0.2024	-0.6190	0.0147	0.2081
1	8.0000	0.2591	0.0804	-0.1827	0.1618	-0.5480	0.0171	0.2219
1	9.0000	0.4420	0.0125	-0.0743	0.4296	-0.8553	0.0000	-0.0143
1	10.0000	0.4543	0.0087	-0.0612	0.4440	-0.8529	0.0020	-0.0808

……

9.4.3 Results files for significant QTL

QAD files: Significant QTL from JICIM (significant additive QTL can be selected from this file; Table 9.11).

- Chromosome: Chromosome ID represented by an integer number.
- Position: The scanning position in cM on the chromosome.
- LeftMarker: Name of the left-side marker of the identified QTL.
- RightMarker: Name of the right-side marker of the identified QTL.
- LOD: joint LOD score of QTL calculated from JICIM.
- *_LOD: LOD score of QTL in each family.
- *_ADD: Additive effect of QTL in each family.

Table 9.11 Significant QTL from JICIM (QAD, incomplete)

Chromosome	Position	LeftMarker	RightMarker	LOD	An_LOD	Kas_LOD	Kond_LOD	An_ADD	Kas_ADD	Kond_ADD
1	66.0000	CIW1	F6D8.94	3.7492	0.6260	0.7903	2.3331	0.5508	-1.1939	2.6380
1	107.0000	SNP157	SNP110	6.1137	4.9961	1.1176	-0.0001	1.4099	-1.3217	-0.0033
2	0.0000	msat2.5	SNP180	3.0477	0.0005	0.0827	2.9647	0.0147	0.3761	2.8658
2	16.0000	SNP184	F12A24b	2.1539	0.0030	0.1194	2.0317	0.0383	0.4852	2.7230
3	0.0000	SNP105	nga172	13.3892	10.4870	0.5984	2.3039	-2.3177	-1.0540	-2.7369
4	3.0000	msat4.41	FRI	43.2310	1.6578	15.9115	25.6616	-0.8231	5.6022	11.2428
4	49.0000	SNP295	SNP199	8.5092	0.0088	7.0913	1.4094	0.0605	3.9011	2.0200
5	19.0000	SNP136	SNP358	13.6721	5.2825	4.5456	3.8441	1.4969	2.7762	3.4790
5	33.0000	SNP236	nga139	5.8686	0.0056	0.6157	5.2474	-0.0494	0.9879	4.1828
5	93.0000	SNP101	SNP304	8.0030	3.1967	4.4736	0.3324	1.1230	-2.7592	1.0146

Chapter 10. Mapping of Segregation Distortion Locus

10.1 Input file for mapping segregation distortion locus (*.sdl)

10.1.1 General information of the mapping population

Six parameters were used for the general information defining a SDL mapping population (Table 10.1).

- Population Type: describe the type of the population. At present, QTL IciMapping can conduct SDL mapping for twenty populations derived from two parental lines (Figure 1.1). Assuming F1 = P1 x P2, the 20 biparental populations are:
  - 1. P1BC1F1: the backcross population where the first parent (P1) is used as the recurrent.
  - 2. P2BC1F1: the backcross population where the second parent (P2) is used as the recurrent.
  - 3. F1DH: doubled haploids derived from F1.
  - 4. RIL: recombination inbred lines derived from repeated selfing since F1 generation.
  - 5. P1BC1RIL: recombination inbred lines derived from the backcross population where the first parent is used as the recurrent.
  - 6. P2BC1RIL: recombination inbred lines derived from the backcross population where the second parent is used as the recurrent.
  - 7. F2: the selfing generation of F1.
  - 8. F3: the selfing generation of F2.
  - 9. P1BC2F1: the second backcrossing where P1 is used as the recurrent parent.
  - 10. P2BC2F1: the second backcrossing where P2 is used as the recurrent parent.
  - 11. P1BC2RIL: recombination inred lines through the repeated selfing of P1BC2F1.
  - 12. P2BC2RIL: recombination inred lines through the repeated selfing of P2BC2F1.
  - 13. P1BC1F2: the selfing generation of P1BC1F1.
  - 14. P2BC1F2: the selfing generation of P2BC1F1.
  - 15. P1BC2F2: the selfing generation of P1BC2F1.
  - 16. P2BC2F2: the selfing generation of P2BC2F1.
  - 17. P1BC1DH: P1BC1F1-derived doubled haploids.
  - 18. P2BC1DH: P2BC1F1-derived doubled haploids.
  - 19. P1BC2DH: P1BC2F1-derived doubled haploids.
  - 20. P2BC2DH: P2BC2F1-derived doubled haploids.
- Mapping Function: specify the mapping function which will be used to transfer recombination frequency to mapping distance, or from mapping distance to recombination frequency.

- 1 for Kosombi mapping function.
- 2 for Haldane mapping function.
- 3 for Morgan mapping function.

- Marker Space Type: specify whether the markers on a chromosome (or linkage group) are defined by positions or marker intervals.
  - 1 for intervals, i.e. the number behind a marker is the distance of the marker to its next marker. 0 is normally given for the last marker on a chromosome or a linkage group.
  - 2 for positions, i.e. the number behind each marker is the position of the marker on the chromosome or the linkage group.

- Marker Space Unit: specify the unit used in marker linkage group.
  - 1 for centi-Morgan (cM).
  - 2 for Morgan (M). 1 M = 100 cM.

- Number of Chromosomes: specify the number of chromosomes (or linkage groups) in the mapping population.

- Population Size: number of individuals in the mapping population.

Table 10.1 General information in a SDL mapping input file

```
!***** Note: lines staring with "!" are remarks and will be ignored in the program***
!***** General Information ************************************************
!Assuming F1 = P1 x P2, populations available in QTL IciMapping are:
!  1, P1BC1F1 = P1 x F1, the first backcrossing where P1 is used as the recurrent parent;
!  2, P2BC1F1 = P2 x F1, the first backcrossing where P2 is used as the recurrent parent;
!  3, F1DH, F1-derived doubled haploids;
!  4, RIL or F1RIL, recombination inbred lines through the repeated selfing of F1;
!  5, P1BC1RIL, recombination inbred lines through the repeated selfing of P1BC1F1;
!  6, P2BC1RIL, recombination inbred lines through the repeated selfing of P2BC1F1;
!  7, F2, the selfing generation of F1;
!  8, F3, the selfing generation of F2;
!  9, P1BC2F1, the second backcrossing where P1 is used as the recurrent parent;
! 10, P2BC2F1, the second backcrossing where P2 is used as the recurrent parent;
! 11, P1BC2RIL, recombination inbred lines through the repeated selfing of P1BC2F1;
! 12, P2BC2RIL, recombination inbred lines through the repeated selfing of P2BC2F1;
! 13, P1BC1F2, the selfing generation of P1BC1F1;
! 14, P2BC1F2, the selfing generation of P2BC1F1;
! 15, P1BC2F2, the selfing generation of P1BC2F1;
! 16, P2BC2F2, the selfing generation of P2BC2F1;
! 17, P1BC1DH, P1BC1F1-derived doubled haploids;
! 18, P2BC1DH, P2BC1F1-derived doubled haploids;
! 19, P1BC2DH, P1BC2F1-derived doubled haploids;
! 20, P2BC2DH, P2BC2F1-derived doubled haploids;
4        !Mapping Population Type ((see remarks above)
2        !Mapping Function (1 for Kosambi Function; 2 for Haldane Function; 3 for Morgan
mapping function)
2        !Marker Space Type (1 for intervals; 2 for positions)
1        !Marker Space Unit(1 for centiMorgans; 2 for Morgan)
12       !Number of Chromosomes (or Linkage Group)
71       !Population Size in the mapping population
```

10.1.2 Linkage group or chromosome information

The name of each chromosome and the number of markers on the chromosome were specified first (Table 10.2), followed by the definition of each chromosome (Table 10.3). Each chromosome was defined by all markers on it and all marker positions.

Table 10.2 Linkage group information in a SDL mapping input file

```
!**********************Information for Chromosomes and Markers**********************
!Chromosome     NumMarkers in each chromosome
-Ch1           36
 -Ch2          31
 -Ch3          31
 -Ch4          22
 -Ch5          17
 -Ch6          13
 -Ch7          16
 -Ch8          17
 -Ch9          13
 -Ch10         16
 -Ch11         24
 -Ch12         14
```

Table 10.3 Definition of each chromosome (incomplete)

```
!Linkage map (Marker name followed by position or the interval length)
C112      1    0.000
XNpb346    1    3.081
XNpb54     1    6.995
R3203      1   12.574
G2200      1   14.814
C86       1   25.244
C3029C     1   41.494
C2340      1   44.575
C813      1   46.068
XNpb343    1   53.568
XNpb393    1   54.326
C466B      1   55.819
C1370      1   60.459
R1012      1   72.282
R886      1   85.053
R1485      1   89.682
XNpb302    1   91.153
R2635      1   95.873
XNpb297-2  1   97.513
R1468C     1   98.320
Y2820R     1   99.791
R1928      1  100.516
R2159      1  101.230
XNpb364    1  105.371
C904      1  107.811
Y5714L     1  108.546
XNpb252    1  110.929
XNpb87-2   1  113.312
R210      1  119.172
C1211      1  123.149
C955      1  140.118
R3192      1  154.382
R1944      1  158.132
R1613      1  160.372
XNpb216    1  168.194
C970      1  168.952
……
XNpb124-1 12   54.958
XNpb189-2 12   69.606
C718B     12   73.714
C104A     12   81.124
XNpb24-2  12   86.562
C562B     12   87.276
```

10.1.3 Marker type information

Marker types are arranged by the ordered markers defined in Table 10.3. That is, the marker types for all individuals on the first marker were given first, then followed by the second marker, and so on (Table 10.4). The marker name in this part has to be the same as that specified in Table 10.3. In IciMapping, 2 was used to represent the marker type of the first parent (P1), 0 for the second parent (P2), 1 for the F1 marker type, and -1 for any missing markers. Any missing markers will be assigned values based on the types of their neighboring markers. The number of marker types behind each marker name has to be exact the population size.

Table 10.4 Marker type information in a SDL mapping input file (incomplete)

```
!**********************************Marker Type**************************************
!Marker type: 2 for P1; 1 for F1; 0 for P2; BC1=F1xP1; BC2=F1xP2; -1 for missing markers if any.
C112       0  2  0  0  0  2  2  0  2  2  0  2  0  2  2  0  0  2  0  2  2  2  0  2  2  0  2  0  0
0  2  0  2  2  0  0  0  0  0  2  0  0  0  0  2  2  0  0  2  0  0  0  0  0  2  2  2  2  0  0  0  0
0  2  0  2  0  0  0  2  0
XNpb346     0  2  0  0  0  0  2  0  2  2  0  2  0  2  2  0  0  2  0  2  2  2  0  2  2  0  2  0
0  0  2  2  2  2  0  0 -1  0  0  2  0  0  0  0  2  2  0  0  2  0  0  2  0  0  2  2  0  2  0  0  0
0  0  2  0 -1  0  0  0  2  0
XNpb54      0  2  0  0  0  0  0  0  2  2  0  2  0  2  2  0  0  2  0  2  0  2  0  2  2  0  2  0
0  0  2  2  2  2  0  0  2  0  0  2  0  0  0  0  2  2  0  0  0  0  0  2  2  0  0  2  0  2  0  0  0
0  0  2  0  0  0  0  0  2  0
R3203       2  2  0  0  0  0  0  0  2  2  2  2  0  2  2  0  0  2  0  2  0  2  0  0  2  0 -1  0  0
0  2  0  2  2  0  0  2  0  0  0  0  0  0  0  2  2  0  0  0  0  0  2  2  0  0  2  2  2  0  0  2  0
0  2  0  0  0  0  0  2  0
G2200       2  2  2  0  0  0  0  0  2  2  2  2  0  2  2  0  0  2  0  2  0  2  0  0  2  0 -1  0  0
0  2  0  2  2  0  0  2  0  0  0  0  0  0  0  2  2  0  0  2  0  0  2  2  0  0  2  2  2  0  0  2  0
0  2  0  0  0  0  0  0  0
C86        2  2  2  0  0  0  0  0  0  0  2  2  0  2  2  0  0  0  0  2  2  2  0  0  2  0 -1  0  0
2  2  0  0  2  0  0  2  0  0  0  0  2  0  0  0  2  2  0  2  0  0  2  2  2  0  2 -1  2  0  0  2  0
0  2  0  0  0  0  0  0  2
……
```

10.2 Input file for SDL mapping in the EXCEL format

One population for SDL mapping in biparental populations can also be defined in an Excel file with the extension name ‘xls’ or ‘xlsx’. The file should be composed of four sheets: ‘GeneralInfo’ (similar to Table 10.1), ‘Chromosome’ (similar to Table 10.2), ‘LinkageMap’ (similar to Table 10.3), and ‘Genotype’ (similar to Table 10.4).

10.3 Setting mapping parameters

The SDL functionality can be initiated by (1) opening input files, or (2) double clicking SDL files listed in the project window, or (3) clicking one SDL file in the display window. When functionality SDL is activated, the Display window shows the contents of the current input file, and the Parameter window is for interaction with users.

Two mapping methods can be conducted in IciMapping, i.e. single marker analysis (SMA), and the traditional inyterval mapping (SIM).

10.3.1 Parmeters for SMA (Single Marker Analysis)

- LOD Threshold: the threshold LOD score to declare significant SDL which is determined by manual input.

10.3.2 Parameters for SIM (Simple Interval Mapping)

- Step (cM): the step in scanning represented by cM.
- LOD Threshold: the threshold LOD score to declare significant SDL which is determined by manual input.

10.4 Outputs

For SDL, the output files have the same prefex name as the input file but with different extension names. Each method (i.e. SMA or SIM) has two kinds of output, denoted as R (for results at any scanning position) and Q (for significant SDL).

10.4.1 Results from all scanning markers or chromsomal positions

1. RSM file: Mapping results from single marker analysis at any tesing positions (Table 10.5)

   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: Marker name, same as given in the SDL input file.
   - Chromosome: Chromosome ID represented by an integer number.
   - Position: Marker position in cM on the chromosome.
   - LOD: LOD score calculated from single marker analysis.
   - Fitness(AA): Fitness of markertype AA. Let n(MAX) be the maximum value of n(AA), 0.5*n(Aa) and n(aa). Fitness(AA) is n (AA)/n (MAX) for marker type AA.
   - Fitness(Aa): Fitness of markertype Aa, i.e. 0.5*n(Aa)/n(MAX).
   - Fitness(aa): Fitness of markertype aa, i.e. n(aa)/n(MAX).
   - n(AA): Observed number of individuals with markertype AA.
   - n(Aa): Observed number of individuals with markertype Aa.
   - n(aa): Observed number of individuals with markertype aa.
   - n(-): Number of missing marker points.

Table 10.5 Results of single marker analysis (RSM, incomplete)

MarkerID	MarkerName	Chromosome	Position	LOD	Fitness(AA)	Fitness(Aa)	Fitness(aa)	n(AA)	n(Aa)	n(aa)	n(-)
1	C112	1	0.0000	0.5198	0.6905	0.0000	1.0000	29	0	42	0
2	XNpb346	1	3.0810	0.5350	0.6829	0.0000	1.0000	28	0	41	2
3	XNpb54	1	6.9950	1.1177	0.5778	0.0000	1.0000	26	0	45	0
4	R3203	1	12.5740	1.0165	0.5909	0.0000	1.0000	26	0	44	1
5	G2200	1	14.8140	0.8012	0.6279	0.0000	1.0000	27	0	43	1

……

2. RIM file: Results from simple interval mapping at any tesing positions (Table 10.6)

- Chromosome: Chromosome ID represented by an integer number.
- Position: The scanning position in cM on the chromosome.
- LOD: LOD score.
- Fitness(AA): Fitness of markertype AA.
- Fitness(Aa): Fitness of markertype Aa.
- Fitness(aa): Fitness of markertype aa.

Table 10.6 Results of simple interval mapping (RIM, incomplete)

Chromosome	Position	LOD	Fitness(AA)	Fitness(Aa)	Fitness(aa)
1	0.0000	0.5198	0.6905	0.0000	1.0000
1	1.0000	0.5116	0.6925	0.0000	1.0000
1	2.0000	0.5062	0.6939	0.0000	1.0000
1	3.0000	0.5035	0.6946	0.0000	1.0000
1	4.0000	0.6754	0.6549	0.0000	1.0000
1	5.0000	0.8036	0.6296	0.0000	1.0000

……

10.4.2 Results files for significant SDL

1. QSM file: All significant segregation distortion markers from single marker analysis (Table 10.7)

   - MarkerID: Marker ID represented by an integer number.
   - MarkerName: Marker name, same as given in the SDL input file.
   - Chromosome: Chromosome ID represented by an integer number.
   - Position: Marker position in cM on the chromosome.
   - LOD: LOD score calculated from single marker analysis.
   - Fitness(AA): Fitness of markertype AA.
   - Fitness(Aa): Fitness of markertype Aa.
   - Fitness(aa): Fitness of markertype aa.
   - n(AA): Observed number of individuals with markertype AA.
   - n(Aa): Observed number of individuals with markertype Aa.
   - n(aa): Observed number of individuals with markertype aa.
   - n(-): Number of missing marker points.

Table 10.7 Significant markers from single marker analysis (QSM, incomplete)

MarkerID	MarkerName	Chromosome	Position	LOD	Fitness(AA)	Fitness(Aa)	Fitness(aa)	n(AA)	n(Aa)	n(aa)	n(-)
31	C955	1	140.1180	6.9255	1.0000	0.0000	0.2105	57	0	12	2
32	R3192	1	154.3820	2.5015	1.0000	0.0000	0.4286	49	0	21	1
83	C1452	3	89.2270	2.6489	1.0000	0.0000	0.4200	50	0	21	0

……

2. QIM file: Significant SDL from simple interval mapping (significant SDL can be selected from this file; Table 10.8).

   - Chromosome: Chromosome ID represented by an integer number.
   - Position: The scanning position in cM on the chromosome.
   - LeftMarker: Name of the left-side marker of the identified SDL.
   - RightMarker: Name of the right-side marker of the identified SDL.

- LOD: LOD score.
- Fitness(AA): Fitness of markertype AA.
- Fitness(Aa): Fitness of markertype Aa.
- Fitness(aa): Fitness of markertype aa.

Table 10.8 Significant SDL from SIM (QIM)

Chromosome	Position	LeftMarker	RightMarker	LOD	Fitness(AA)	Fitness(Aa)	Fitness(aa)
1	141.0000	C955	R3192	3.5813	1.0000	0.0000	0.3583
3	89.0000	C361	C1452	2.6327	1.0000	0.0000	0.4212
12	12.0000	R496	C1069	4.2377	1.0000	0.0000	0.3229
12	81.0000	C718B	C104A	7.9605	1.0000	0.0000	0.1865

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