Windows QTL Cartographer 2.5
User Manual

Table of Contents

WinQTLCart Procedures 25

Tutorials 81

About Windows QTL Cartographer

WinQTLCart features

Windows QTL Cartographer maps quantitative trait loci (QTL) in cross populations from inbred lines. WinQTLCart includes a powerful graphic tool for presenting mapping results and can import and export data in a variety of formats.

WinQTLCart incorporates many of the modules found in its command-line sibling, QTL Cartographer, and provides a graphical interface to many of QTL Cartographer's features.

WinQTLCart implements the following statistical methods:

- Single-marker analysis 55
- Interval mapping 58
- Composite interval mapping 60
- Bayesian interval mapping 76
- Multiple interval mapping 63
- Multiple trait analysis 78
- Multiple trait MIM analysis 78

Features

- Supports various QTL mapping methods
- View, copy, and print graphs
- Includes an interface to help you build a source data file that WinQTLCart can use for analysis
- Import 25 data from Mapmaker / QTL and Microsoft Excel and CSV formats
- Export 29 graph data to Windows Excel format
- View, copy, and print chromosome information 78 graphically
- Produce a simulation 51 data file

Compatible programs and formats

WinQTLCart can import and export data files in a variety of formats.
Import success depends on the data file's format. Some data may need to be formatted manually before WinQTLCart can import it.

Applications	Formats Supported	Import	Export
MapMaker/QTL	.MAP – Map file .MPS – Map file .RAW – Cross data file	X	
Microsoft Excel	.XLS	X	X
Microsoft CSV	.CSV	X	
QTL Cartographer	.INP – Map and Cross data files .MAP – Map file .CRO – Cross data file	X	X
WinQTLCart	.MCD – Source data file	X	X

Related topics
Creating a new source data file 44
Troubleshooting import errors 86
Importing files 25
Exporting source data and results 29

System requirements

- WinQTLCart can run on the following operating systems: Windows 95, 98, ME, NT, 2000, XP and Windows 7.
- Because some WinQTLCart windows are quite large, the suggested minimum monitor resolution is 1024x768.
- 20MB free disk space for program files.
- 512MB RAM.
- Any mouse or pointing device supported by Windows.

Installing, uninstalling, upgrading

Installing

To install, double-click the WQTLSetup.exe file and follow the prompts. The default install directory is C:\NCSU\WinQTLCart2.5, though you can specify a different directory.

The installer places a shortcut to the Windows QTL Cartographer program on your PC's Desktop, labeled WinQTLCart. Double-click the icon to run the program.

Uninstalling

To uninstall, run the Add/Remove Programs control panel and select Windows QTL Cartographer from the installed programs list.

Upgrading

If you have a prior version of WinQTLCart already on your PC, simply run the installer program. Upgrading to a new version of WinQTLCart does not overwrite your working files. However, the upgrade will replace the sample files that are part of the WinQTLCart distribution.

Note

You should close current running version of WinQTLCart first before installing the upgrading version.

Using WinQTL - a high-level overview

Your goals in using WinQTLCart may include preparing data for publication or continued research into possible QTL sites.

Step 1—Preparing your source data
Your data files may come from another program or they may exist as raw data files. For WinQTLCart to work with your files, they need to conform to the program's .MCD file format 39. Review that file, as well as the other files included in the WinQTLCart distribution, such as the .QRT, .QPE, and other files.

These are all text files that you can view in any text editor.

Or, you may not have any data files or any data ready for import. You may instead want to use WinQTLCart to create simulation data to try out some hypotheses to view potential results.

See these topics for more information: MCD file format 39, Creating a new source data file from raw data 44, Creating simulation data 51

Step 2—Bringing data into WinQTLCart
WinQTLCart can import map and cross data files from MapMaker/QTL, QTL Cartographer, and Microsoft Excel. As part of the import, WinQTLCart runs verification checks against the data. If the data does not conform to the accepted format, WinQTLCart displays an error message that should indicate the source of the problem.

See these topics for more information: Importing files 25, WinQTLCart cannot import Map information from selected file 86, Invalid file or wrong format messages 86

Your source data may not have come from another program, but may instead exist as raw source files. In that case, using WinQTLCart's Create a New Source File command steps you through all of the steps needed to translate the raw data into a readable form. The new source file will conform to WinQTLCart's MCD file format.

See these topics for more information: Creating a new source data file from raw data 44, MCD file format 39

Step 3—Analyzing data using QTL Mapping Methods
With WinQTLCart able to view the data, you can then select any of seven different analysis methods. The end result for some of these methods is another MCD file, but in most cases the process will create a .QRT result file that WinQTLCart can use to graph QTL information.

See these topics for more information: Single-marker analysis 55, Interval Mapping 58, Composite Interval Mapping 60, Multiple Interval Mapping 63, Bayesian Interval Mapping 76, Multiple-trait Analysis 63, Multiple-trait MIM.

Step 4—Viewing results and graphs
WinQTLCart can present your data in graphics suitable for publication. You can show all chromosomes and their intervals in one display, while the Graph window display offers many parameters to help you fine-tune the visualization.

See these topics for more information: Drawing a chromosome tree 78, Graph Window tour 17

Step 5—Saving and exporting results
You can save your source data in .MCD format and your results files in .QRT format so you can work with them later in WinQTLCart. You can also export your results to other selected formats.

See these topics for more information: Exporting source data and results 29, Exporting results from the Graph window 31, Exporting source data to an MCD file 30, Exporting source data to QTL Cartographer 30

When to use WinQTLCart

You can use WinQTLCart for any kind of data that is cross populations from inbred lines. WinQTLCart is a particularly powerful tool when you want to explore your results graphically.

Prior to doing experiments, you could use WinQTLCart to explore some "what-if" scenarios in planning your experimental design. In WinQTLCart, you can create simulation data and then vary parameters setting to explore various QTL models.

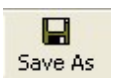
However, if you're working on a repetitive task that would be better off scripted, you may want to turn to QTL Cartographer, WinQTLCart's command-line sibling. For example, if you have expression data with thousands of features, you might want to run interval mapping on each feature, which would take a long time. This task can be automated via a shell script and made to run overnight. The results can then be imported into WinQTLCart and its graphs charted.

WinQTLCart Windows & Menus

Main window - Menus

Menus in Main window include File 4, Edit 5, View 5, Method 5, Tools 6, and Help 7.

Main window - Menus - File

Icon	Command	Shortcut Key	Function
	New...	Ctrl + N	Create a new source data file from raw text files. See Creating a new source data file 44
	Open...	Ctrl + O	Open a data file, result file or text file. Source data files you can open in WinQTLCart have the .MCD extension. Results files have the .QRT extension. Files with any other extension are opened as text files.
	Close		Close the currently selected file. (The currently selected .MCD file's name is in the title bar and highlighted in the Tree pane).
	Save As...		Save the currently active source data to a file with mcd format or a file with Microsoft Excel format.
	Import...	Ctrl + I	Import files in a variety of formats. See the importing files 25 topic
	Simulation...		Opens the Simulate Data dialog. See creating a simulation data file 51
	Export...		Export the selected file to a different format. See Exporting source data and results 29

	Print...	Ctrl + P	Print the selected file in data pane.
	Print Setup...		Opens Windows' Print Setup dialog box.
	Recently used files		WinQTLCart displays the last 6 data files you've worked with.
	Exit		Closes WinQTLCart. If you have unsaved data, you'll be prompted to save it.

Main window - Menus - Edit

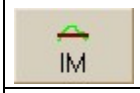
Command	Shortcut Key	Function
Copy	Ctrl + C	Copy selected text in Data pane 12 to the Windows clipboard.
Select All	Ctrl + A	Click in the Data pane 12 and then choose this command to select all text in the data pane. Enables you to easily select and copy the text to a separate file.

Main window - Menus - View

Icon	Command	Shortcut Key	Function
	Data Summary		Summarizes statistical information of active source data file and displays in Text window.
	Result Graph...	Ctrl + G	View result file in the Graph window. See Graph Window tour 17
	Toolbar		Select to toggle the Toolbar display.
	Status bar		Select to toggle the Status bar display.

Main window - Menus - Method

Icon	Command	Function
	Single Marker Analysis...	Displays the Single Marker Analysis 55 form.

	Interval Mapping...	Displays the Interval Mapping 58 form.
	Composite Interval Mapping...	Displays the Composite Interval Mapping form 60.
	Multiple Interval Mapping...	Displays the Multiple Interval Mapping 63 form. Click "OK" button to start MIM analysis and click "MtMIM" button to choose multiple-trait MIM analysis.
	Multiple Trait MIM Analysis...	Display the Multiple Trait MIM Analysis form.
	Multiple Traits IM-CIM Analysis...	Displays the Multiple Trait Analysis 78 form.
	Category Trait Analysis...	Displays the Category Trait Analysis form.
	Bayesian Interval Mapping...	Displays the Bayesian Interval Mapping 76 form.
	eQTL MIM Analysis...	Displays the eQTL MIM Analysis form.

Main window - Menus - Tools

Icon	Command	Shortcut Key	Function
	Set Working directory...		Set the default working directory. See Setting the working directory 25
	Draw chromosome graph...		Show and print graphic displays of chromosomes from the Current active .MCD file. See Drawing a chromosome tree 78
	Copy between trait and otrait...		Copy normal trait to Other trait (category trait) or vise verse.
	Delete markers of same position...		Delete markers that have the same position in a chromosome and only keep one marker.
	Notepad...	Ctrl + Shift + N	Opens Notepad. Use Notepad as a convenient text editor to help format source data files.
	Calculator...	Ctrl + Shift + C	Open the Windows Calculator accessory.

Main window - Menus - Help

Icon	Command	Function
	About WinQTLCart	Display About dialog of WinQTLCart. You can open WinQTLCart upgrade site in this dialog.

Chromosome graph display - Menus

Open a mcd source data file. From the Main window, select Tools>DrawChrom or click  to draw the trees of chromosome graph 78 and markers in a single large window that is suitable for copying to an image program for later editing or printing and publication.

Menus in Chromosome graph display window include File 7, View 7, Setting 8, and Copy_Graph that will Copy content in window to the clipboard.

Chromosome graph - Menus - File

Command	Function
Copy to Clipboard	Copies content in window to the clipboard.
Print Graph…	Print the graph.
Exit	Close the window and return to the Main window.

Chromosome graph - Menus - View

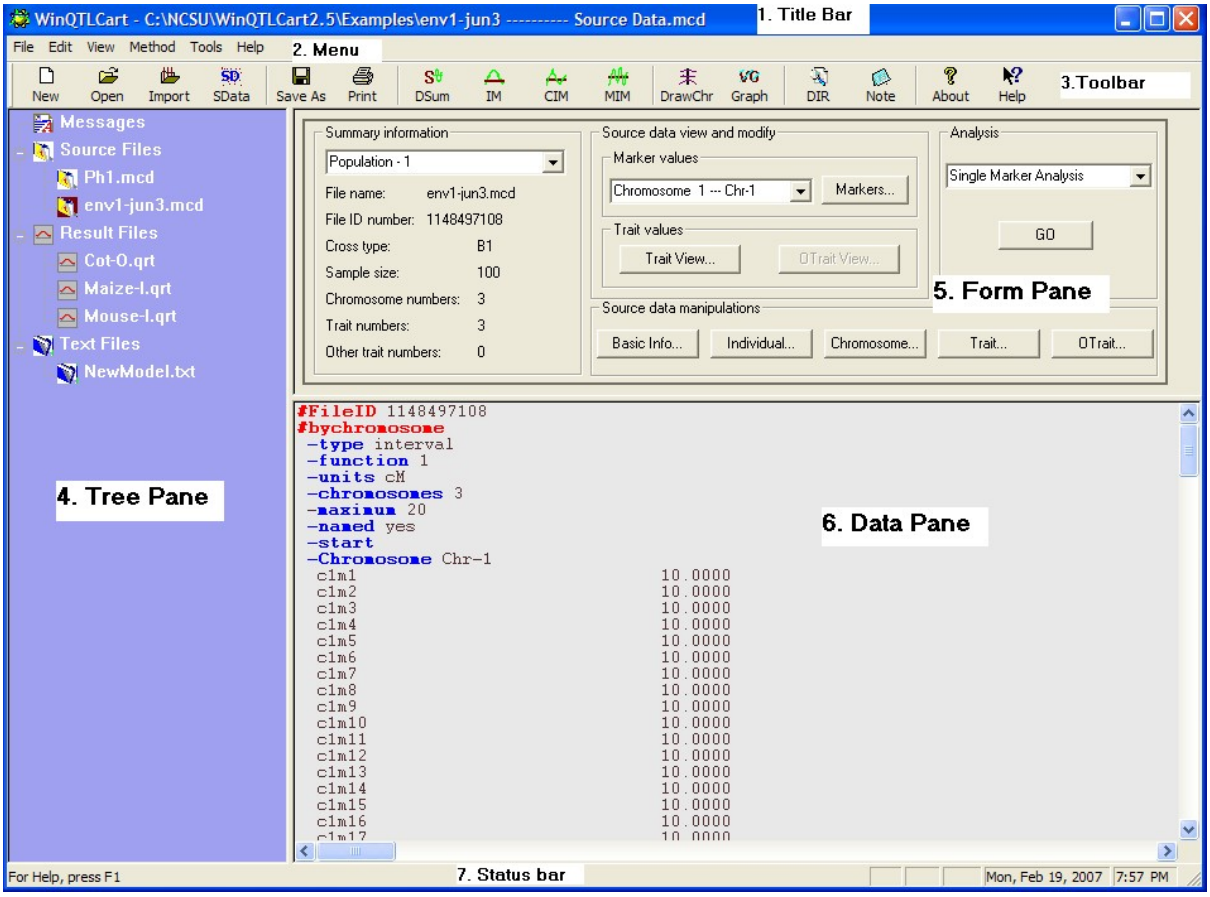
Command	Function
Proportion of Marker Number	Show length of chromosome graph in proportion of marker number
Proportion of Chromosome Len	Show length of chromosome graph in proportion of chromosome length in cM
Next Page >>	Show next page of the graph if there are multiple pages.
First Page	Show First page of the graph.
Add QTL Positions...	Display QTL positions in the graph.

Chromosome graph - Menus - Setting

Command	Function
Select Chromosomes	Select chromosomes to be showed in graph.
Show Chromosome Name	Toggle display between chromosome names or chromosome labels produced by WinQTLCart.
Font Size >>	Increase font size of graph.
Font Size <<	Decrease font size of graph.
Space Between >>	Increase space between chromosomes.
Space Between <<	Decrease space between chromosomes.
Chromosome Name >>	Increase font size of chromosome names.
Chromosome Name <<	Decrease font size of chromosome names.
Column Number >>	Increase the number of chromosome displayed horizontally.
Column Number <<	Decrease the number of chromosome displayed horizontally.

Main window tour

When you start up Windows QTL cartographer, the program's Main window displays.



From top to bottom, here's what you see:

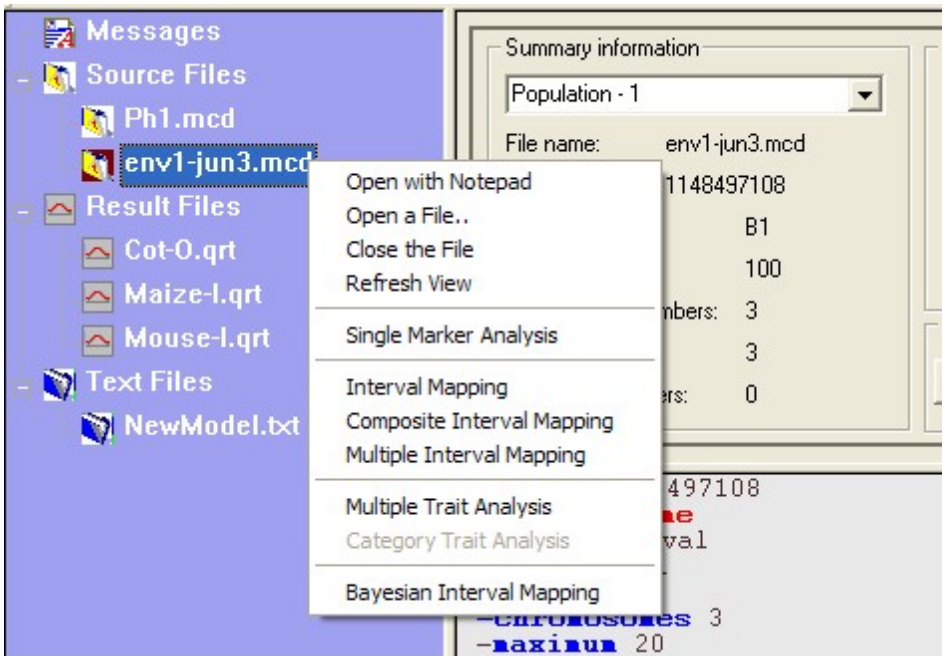
1. Title bar. Shows the name of the selected source data file.
2. Menu bar 4.
3. Toolbar with one-click access to the program's major functions. Hover the pointer over a button to see a brief description of that command. The button's function is also described in the Status bar at the bottom of the window.
4. Tree pane 10 for file management. Lists open files and organizes files under various category names (Source Files, Text Files, Results Files).
5. Form pane 12 for displaying and controlling analysis of source data files. The form pane contents change based on the analysis method you select.
6. Data pane 12 for displaying data of currently selected file.
7. The Status bar displays a variety of system messages; select View>Status bar to toggle its display. Click on each node in the tree pane or hover the pointer over a toolbar button or menu command to see the displayed message. The right area of the status bar also displays the current date and time, and also displays CAP, NUM, or SCRL if the Caps Lock, Num Lock, or Scroll Lock keys have been pressed.

- Double-click on a category name in the Tree pane to open files of that type.
- Left-click on a .MCD filename to make that the active source data file on which to run an

analysis.
- Right-click on a filename to see appropriate commands for that file.

Main window - Tree Pane

The Main Window's Tree pane allows you to manage open files. The following table describes the many different options available via left-click, right-click, and double-click operations.



Source file selected with right-click options displayed

Tree Item	Action	Function
Message window	Left-click	Displays WinQTLCart startup message in the data pane.

Tree Item	Action	Function
Source files root	Double-click	Open a source data file (.MCD)
	Right-click>Open a File	Open a source data file (.MCD)

Tree Item	Action	Function
Result files root	Double-click	Open a result file (.QRT)
	Right-click>Open File	Open a result file

Tree Item	Action	Function
Text files root	Double-click	Open a text file (.TXT).
	Right-click>Open File	Open a text file

Tree Item	Action	Function
.MCD file	Left-click	Show file contents in the Data pane and set as current working .MCD file
	Double-click	Open the .MCD file in Notepad
	Right-click options…	
	>Open File	Open a new source data file
	>Open with Notepad	Open the .MCD file in Notepad
	>Refresh	Re-load the file after modification
	>Single Marker	Start single marker analysis
	>Interval Mapping	Start interval mapping
	>Composite IM	Start composite interval mapping
	>Multiple Traits	Start multiple traits analysis
	>Multiple IM	Start multiple interval mapping
	>Bayesian IM	Start Bayesian interval mapping

Tree Item	Action	Function
.QRT file	Left-click	Show this result file in text format via the Data pane 12.
	Double-click	Open the QRT file in the Graph window 17
	Right-click options…	
	>Open File	Open a new result file
	>Open with Notepad	Open the .QRT file with Notepad
	>Refresh	Re-load the file after modification
	>Close File	Close the result file
	>Graphic Dialog	Open the .QRT file in the Graph window

Tree Item	Action	Function
.TXT file	Left-click	Show this text file in the Data pane 12
	Double-click	Open the text file with Notepad
	Right-click options…	
	>Open File	Open a new text file
	>Open with Notepad	Open the .TXT file with Notepad
	>Refresh	Re-load the file after modification
	>Close File	Close the text file.

Main window - Form Pane

The Form pane is the control panel you use to analyze your source data. It serves as a dashboard that presents a lot of information about your source data file at a glance. (The Form pane is keyed to the .MCD source data file only; it does not show information for any other file format.)

This "control panel" changes based on the analysis method you select. For each analysis method, WinQTLCart displays different parameters and controls that help you control the analysis.

When you first open WinQTLCart, you see the standard Source Data File Information form. Most of the options are disabled because no source data file has been loaded. Select an analysis method from the drop down list in the Analysis box on the right to begin working with the data.

When you have opened a file, WinQTLCart enables the buttons and controls on the Source Data File Information form. These enable you to perform some basic manipulations to the source data (such as add traits, map information, etc.)

Forms and disabled commands
When you select an analysis method, WinQTLCart assumes you want to keep working with that method until you save your data or cancel the analysis. If you select the Interval Mapping (IM) method, WinQTLCart disables several toolbar and menu commands (such as the other analysis methods, setting the working directory, and so on). You need to either save your data or press the Cancel button on the IM form to leave the IM analysis mode. Leaving an analysis method re-displays the Source Data File Information pane.

See the Source data file information 31 topic and the topics for each analysis method for the appropriate screen shot relevant to that method.

Main window - Data Pane

The large pane under the Form pane 12 displays the content of the active data or results file in text format. You cannot edit the displayed information from this pane. However, you can select the text with your cursor and copy the selected text to the clipboard.

For .MCD source data files, WinQTLCart color-codes the data so you can easily determine what are comments, labels, headers, and so on.

Graph window - Menus

From the Main window, select View>Visualize Result to display the result file (*.qrt) in result graph window.

Menus in Graph window include File 13, Chrom 13, Traits 14, Effects 14, Tools 15, and Setting 15.

In addition to the toolbar and menu commands, some functions are available by right-clicking on the graph.

Graph window - Menus - File

	Command	Function
	Open QTL Result File...	Open a result file. Files with the .QRT extension are considered result files.
	Add QTL Result Graph...	Adds a new graph to the current display. Files with the .QRT extension are considered result files. Note: The added result file should have same chromosome number and marker number as original one. You could add more than one new graph.
	Copy Graphic to Clipboard	Copies the graph to the Windows clipboard.
	Save As New Name...	Save the file under a different name in .QRT format. You may want to do this if you plan to work with the results in a later WinQTLCart session.
	Save As Text File...	Save the results as a text file. You may want to do this if you plan to use the text file in another program.
	Save As Excel File...	Save the data as an Excel file. Use Excel's charting capabilities to draw the graph.
	Save As EQTL File..	Save the data as the EQTL format that is used on Command-line version of QTL Cartographer.
	Print Graph...	Print the graph to a selected printer.
	Exit	Closes the Graph window. If you have unsaved data, you'll be prompted to save it.

Graph window - Menus - Chrom

	Command	Function
	Next Chrom >>	Display the next chromosome in the file.
	Prev Chrom <<	Display the previous chromosome in the file.
	Select Chroms…	Choose the chromosomes you want to graph. You can also change the order of the chromosome display. See Selecting chromosomes for graph display.
	Show All Chroms	Shows all chromosomes in the file in a single graph.

Graph window - Menus - Traits

	Command	Function
	Next Trait >>	Display the next trait in the file.
	Prev Trait <<	Display the previous trait in the file.
	Select Trait(s)…	Choose the traits you want to graph. See Selecting traits for graph display 20.
	Show All Traits	Shows all traits in the file in a single graph.

Graph window - Menus - Effects

	Command	Function
	Show Additive Effect	Display additive effect graph at bottom of window. This is on by default for new Graph windows.
	Show Dominant Effect	Display dominant effect graph at bottom of window if cross type is SF_n or RF_n.
	Show Values of R2	Display R2 value graph at bottom of window.
	Show Values of TR2	Display TR2 value graph at bottom of window.
	Show Values of S	Display S value graph at bottom of window.
	One Standard Deviation	Normalize the additive and dominant effect in one standard deviation.

Graph window - Menus - Tools

	Command	Function
	Display One Page Format...	Show the graph information in a smaller, one-page format, for publication purposes. See One-page display window - Menus 16 for more information.
	Show QTLs information...	Display QTL information from a simulation parameter file or summary QTL peaks. See Showing QTL information 24.

Graph window - Menus - Setting

	Command	Function
	Set Display Parameters…	Allows you to customize the graph display. See Setting display parameters 21.
	Set Test Hypothesis…	Display result of different tests, such as H1:H3. See Setting a test hypothesis. 23
	Show Graph in LR/LOD Scale	Toggles between LR and LOD scale displays. Look for LR or LOD at the top of the y-axis line.
	Show Black and White Graph	Toggle between color and black-and-white display. Use black-and-white graphs for publication.
	Show Colorful Background	Activate a color or white background; might be useful for printing or to provide better contrast for color graph lines.
	Hide/Show Threshold Lines	Toggle display.
	Show Horizontal Grids	Toggle display.
	Show Vertical Grids	Toggle display.
	Show Trait Names or Legend	Toggle display. If traits are present, WinQTLCart defaults to showing legends on the right side of the graph. Use Set Display Parameters 21 to switch the legends to above the graph. Turn the trait name display on when you're loading more than one result file into a graph.
	Show Marker Names	Toggle display.
	Show Chromosome Names	Toggle display.

	Trace Coordinate in Graph	Provides coordinates for a specific point on the graph. See Tracing coordinates on the graph 19.

One-page display window - Menus

From the Graph window, select Tools>Display One Page Format to display the graphs in a single, large, scrolling window that is suitable for copying to an image program for later editing or printing for publication.

The chromosomes and traits shown in the one-page display depends on the Select Chroms 21 and Select Traits 20 settings in the Graph window.

Menus in One-page display window include File 16, View 16, Setting 17, and Copy_Graph that will Copy content in window to the clipboard.

One-Page window - Menus - File

Command	Function
Copy to Clipboard	Copies content in window to the clipboard.
Print Graph…	Print the graph.
Quit	Close the window and return to the Graph window.

One-Page window - Menus - View

Command	Function
Show Frame	Puts a border around the graph(s).
LR Proportion	Graph heights according to LR values.
Show Color Graphic	Toggle display of colors (axis lines remain black).
Show Threshold Line	Toggle display of threshold line.
Show Marker Number	Toggle display of marker numbers.
Row Number>>	Increase graph number in a row by 1.

Row Number<<	Decrease graph number in a row by 1.
Column Number>>	Increase graph number in a column by 1.
Column Number<<	Decrease graph number in a column by 1.

One-Page window - Menus - Setting

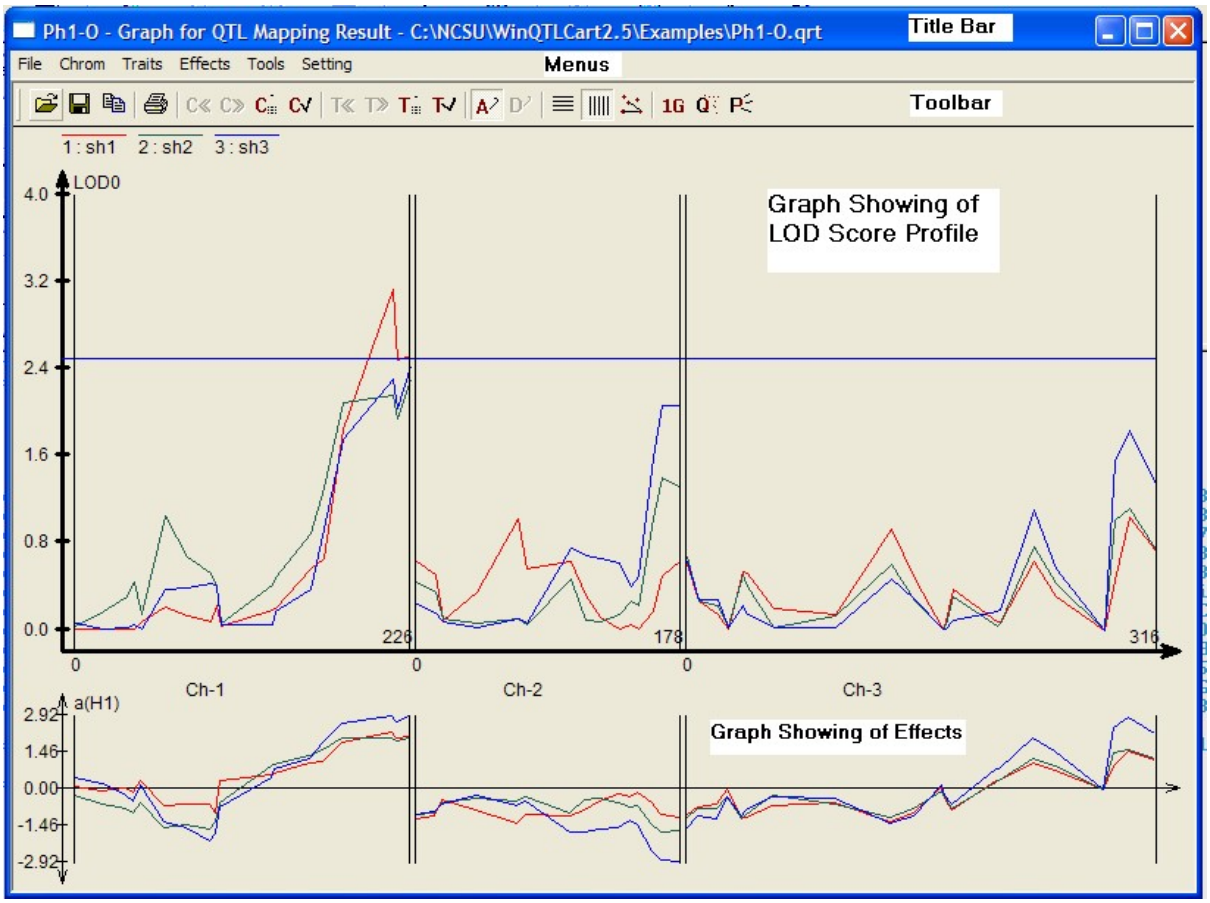
Command	**Function**
HSpace Between >>	Increase horizontal space between chromosomes.
HSpace Between <<	Decrease horizontal space between chromosomes.
VSpace Between >>	Increase vertical space between chromosomes.
VSpace Between <<	Decrease vertical space between chromosomes.
Title Font Size >>	Increase title size.
Title Font Size <<	Decrease title size.

Graph window - Procedures

To see the Graph window, open a WinQTLCart results file (with a .QRT extension) by click the Result button on the Main window's toolbar. The Graph window opens, presenting a graphical overview of the results file data.

From this window you can:

- Spot the location of QTLs. A graph peak that extends past the threshold line is the site of a QTL.
- Load multiple results files at one time and compare them. This could be useful when comparing the results of the same dataset pushed through different analysis methods and parameters.



From top to bottom, here's what you see:

1. Title bar. Shows the name of the selected results data file. You can have multiple results files loaded and multiple Graph windows open at a time.
2. Menu bar 12
3. Toolbar with one-click access to the program's major functions. Hover the pointer over a button to see a brief description of that command.
4. The large graph charts the data as a LOD (or LR) score profile. The higher the LOD, the greater the evidence for a QTL.
5. The smaller graph at the bottom is the QTL effects window, showing additive or dominant effects or R2 or TR2 or S values.

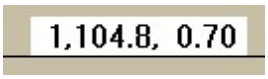
Graph window tips

- You can add several result files so they display at the same time on the current graph. You might want to run your data through the IM, CIM, and MIM analysis methods, for example, and then pull them all into the same graph to see how they compare.
- Peaks above the threshold line indicate a QTL.
- A high LOD value on the graph indicates a good QTL candidate.
- Right-click on the graph to see appropriate commands. (Commands described in the Graph window-Menus 12 topic.)
- You can minimize the Graph window to the bottom of the Main window; a small bit of the title bar is visible.
- You can have the same result file open in several windows at the same time. This might be useful if you're testing various viewing parameters. To do this, minimize the current Graph window, go back to the Main window, ensure the result file is still active, and click the Graph toolbar button.

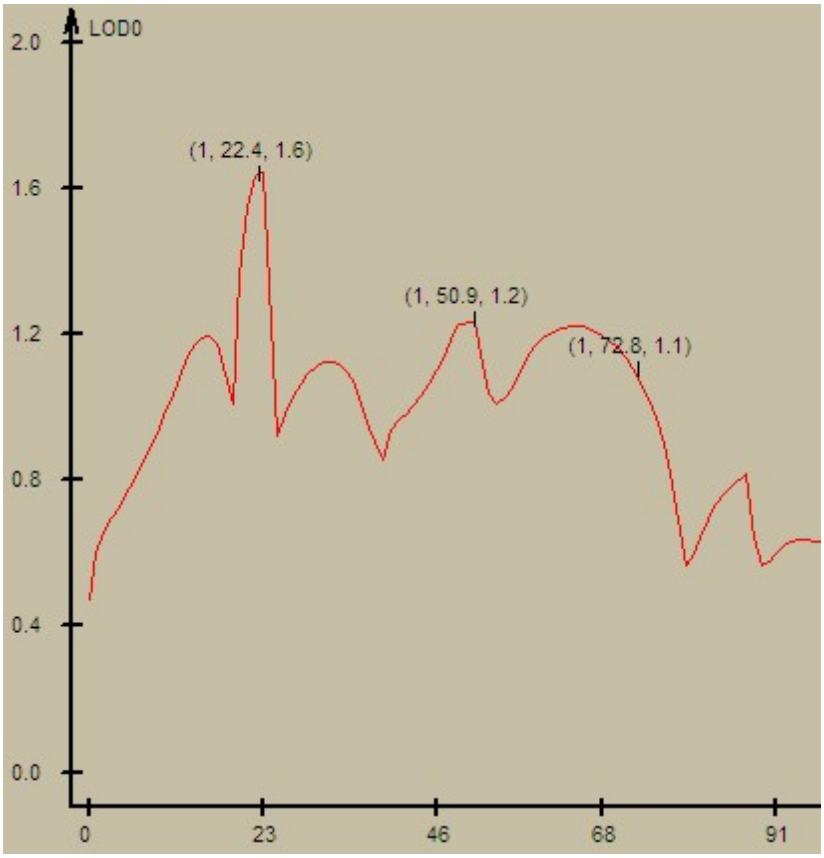
Related topics
Graph Window - Menus 12
Tracing coordinates on the graph 19
Selecting traits for graph display 20
Selecting chromosomes for graph display 21
Setting display parameters 21
Setting a test hypothesis 23
Showing QTL information 24

Tracing coordinates on the graph

Select Settings>Trace Coordinate in Graphic or click  . As you move the cursor around the screen, note that the graph coordinates are displayed in the graph's upper right corner.



Double-click a point on the graph; WinQTLCart marks that point with a dot and displays the coordinates.



Take the following coordinates as an example:

(1, 46.7, 3.0)
1=the chromosome number

46.7=the cM location along the chromosome
3.0=the LOD score

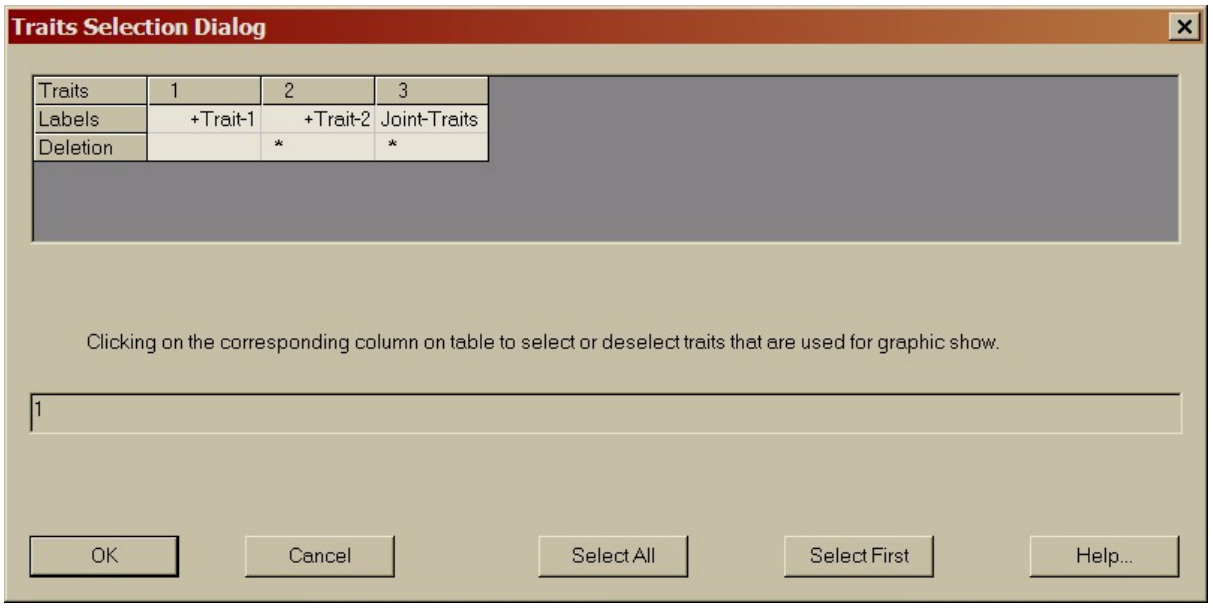
After marking one or more points, copy the graphic to the clipboard (File>Copy to Clipboard) for use in another application or for publication.

Re-select the command to toggle the coordinate display; WinQTLCart also clears from the display the coordinate points you selected.

Related topics
Setting display parameters 21

Selecting traits for graph display

Select Traits>Select Trait(s)… or click  when you want to focus the graph on only a few traits, rather than all of the traits in the data. Selecting the command displays the Select Traits dialog.



Click anywhere in a column to toggle display of the trait in the Graph window.

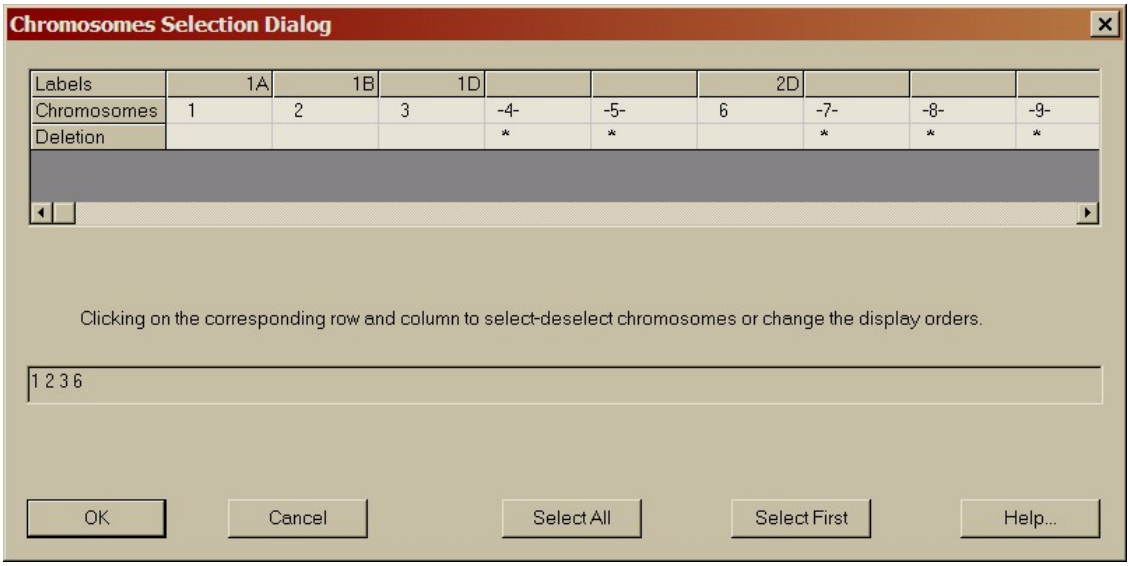
In the screen shot above, the Trait 1 Deletion cell is cleared, meaning this trait ***will*** be displayed. An asterisk in the Deletion cell a trait means it ***will not*** be displayed.

- Click Select All to show all the traits.
- Click Select First to show only the first trait.
- Click Help to display help text for this dialog.

Note *You cannot change the display order of traits. However, you can change the display order of chromosomes.*

Selecting chromosomes for graph display

Select Chrom>Select Chroms… or click  when you want to focus the graph on only a few chromosomes, rather than all of the chromosomes in the data. Selecting the command displays the Select Chromosomes dialog. Use this dialog both to select chromosomes for display and to juggle their display order.



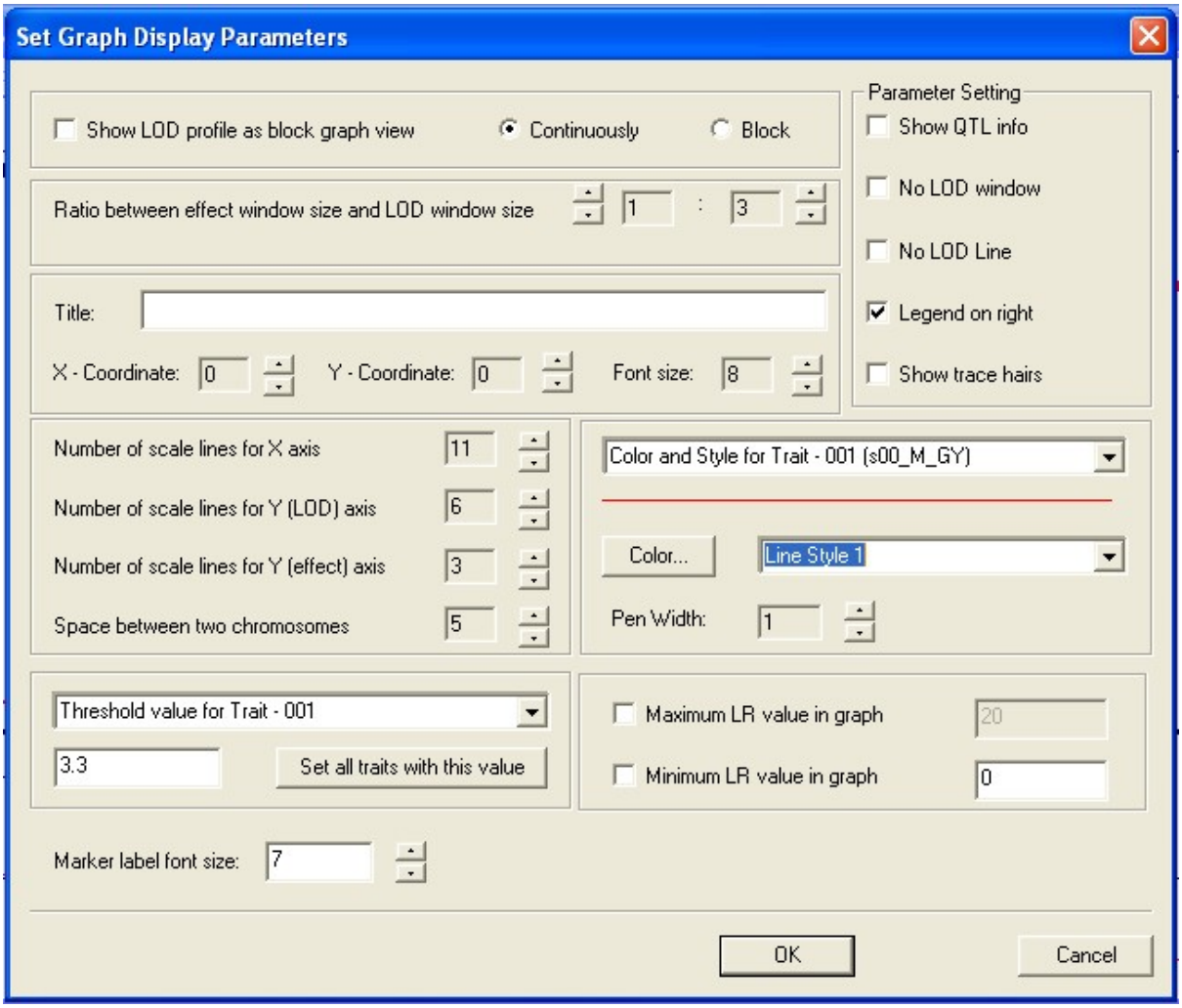
- The lower text field shows the chromosomes and the order in which they will be displayed.
- **To delete a chromosome from the graph display**, click the cell in the Delete row below that chromosome. An asterisk in a field means the chromosome ***will not*** be displayed; a clear field means the chromosome ***will*** be displayed. Click the Deletion cell to toggle display on and off.

  In the screen shot above, the Deletion cells for 1, 2, 3, and 6 are cleared, meaning those chromosomes ***will*** be displayed. When a chromosome is removed from the display, its number disappears from the lower text field, also. In the box above, clicking the empty cell under "3" would remove chromosome chromo3 from the graph display.
- **To reorder chromosomes,** click on a number in the Chromosomes row. It swaps places with the next displayed cell to its right. Clicking the last displayed cell swaps it with the first displayed cell.

  Example: In the screen shot above, clicking 1 will swap 1 and 2, and the display order will be 2 1 3 6. Clicking 3 will swap 3 and 6, so the display order would be 1 2 6 3. Clicking 6 will swap 6 and 1, so the display order would be 6 2 3 1.
- Click Select All to show all the chromosomes.
- Click Select First to show only the first chromosome.
- Click Help to display help text for this dialog.

Setting display parameters

Select Settings>Display Parameters or click  from the toolbar to display the Set Graph Display Parameters dialog. Use this dialog to refine the display, change font sizes and colors used, and so on.



Show LOD profile as block graph view. Check to show color block for LOD / LR profile instead of line curve. Use continuously or block radio button to set the total colors in color block display.

Ratio between effect window size and LOD window size. Use the spin dials to affect the display ratio. You can choose, for example, to make the LOD window the same size as the effect window by selecting 1:1. By default, the LOD window is 3 times the effects window size.

Title. Enter a title for the graph display. Use the X and Y coordinate boxes and the Font size box to precisely place the title so it looks as you want.

Show QTL info. Check to toggle display of QTL information. See Showing QTL information 24 for more information.

No LOD window. Check to suppress display of the LOD / LR graph window (upper window) and only show the effect window.

No LOD line. Check to suppress display of the LOD / LR line curve in upper window.

Legend on right. Check to show the legends to the right of the graphs. Uncheck to show the legends above the graphs.

Show trace hairs. By default, WinQTLCart will not show X and Y cross hairs when you select use the Trace Position command. Check this box if you do want to see the cross hairs.

Number of scale lines for X axis. Specify the number of hash marks spread across the cM scale of the graph.

Number of scale lines for Y (LOD) axis. Specify the number of hash marks spread along the LOD scale.

Number of scale lines for Y (effect) axis. Specify the number of hash marks spread along the effect scale of the graph.

Space between two chromosomes. Specify a distance as a percentage of the graph scale to separate the chromosomes in the graph. (Put in about 5 or 10 to see the effect.)

Threshold value for traits. Select a trait from the drop down list and enter a number to set as that trait's threshold value. Click the Set all traits with this value button to impose a consistent threshold on all displayed traits.

Trait color and line styles. Select a trait from the drop down list. Press the Color button to select its color; select a Line Style from the drop down list to further differentiate it from other traits in the display.

Maximum LR value in graph. Check and input a value to limit the max LR (not LOD) value into the value for the LOD / LR curve line, default value is max LR value in the selected chromosomes and traits.

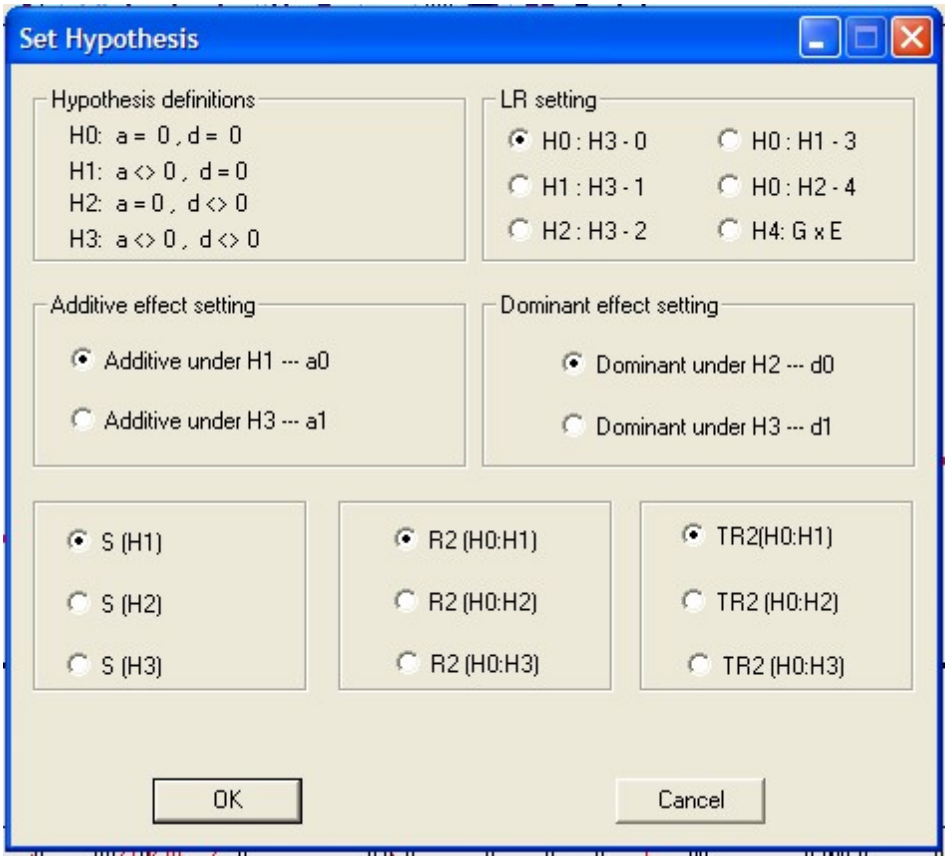
Minimum LR value in graph. Check and input a value to set the minimum LR (not LOD) value into the value at Y-axis, default value is 8.0.

Marker label font size. To adjust marker label's font size after selecting showing marker label in LOD / LR window.

Setting a test hypothesis

Select Setting>Test Hypothesis to play with the results further by trying out different LOD / LR and effects (additive, dominant, R2, TR2, S) settings on the displayed results.

Note: This option is only for crosses with three kinds of genotype such as SF2.



This dialog is fairly self-explanatory. The Hypothesis definitions box describes the conditions for each hypothesis, with a=additive and d=dominant. The LR setting box describes pre-set likelihood ratio hypotheses.

Click OK to apply the selected hypotheses options to your display.

Showing QTL information

Select Effects>Show QTL Information… or click  on the toolbar to display the Show QTL Information dialog. From here, you can show QTLs from a simulation parameter file or show summary QTL information from the likelihood ratio graph peaks.

Select the **Open QTL** information file option and then the Browse… button to select a file that has the QTL positions and effects settings you want to use for the display.

Select the **Show one or two LOD interval** options, as desired, to show empiric QTL confidence intervals—95 percent is one LOD and 99 percent is two LOD.

Select the **Automatically locate QTLs** option to specify parameters WinQTLCart will use to find QTLs in the results. Use the spin dials to specify the minimum acceptable cM range that defines a QTL peak; if the peak's distance is less than this value, then the highest peak will be considered a QTL. The minimum acceptable LOD scale as measured by the highest and lowest points of a QTL peak on the graph. Both of these requirements must be met for a peak to be considered a QTL.

Related topics
Creating simulation data 51

WinQTLCart Procedures

Setting the working directory

By default, WinQTLCart looks for data files and other working files in its home directory (typically C:\NCSU\WinQTLCart) or directory of last opened source data (mcd) file. WinQTLCart saves all files it creates to the current working directory.
But if your data files reside in another directory or on a network drive, you can tell WinQTLCart to look for and save its files there.

1. Select Tools>Set Working Directory to display the Set Working Directory dialog.

2. To change the directory, click Modify…, navigate to the directory you want, and click OK. The new directory appears in the Set Working Directory dialog.

3. Click Set.

Importing and exporting

Importing files

Unless the files you're working on have already been saved as WinQTLCart mapping source data files (files with a .MCD extension) or are already in the .MCD format 39, then you need to import them into WinQTLCart. WinQTLCart will read in the files, verify the data formatting, and save the files in .MCD format automatically.

WinQTLCart can import files from the following applications:

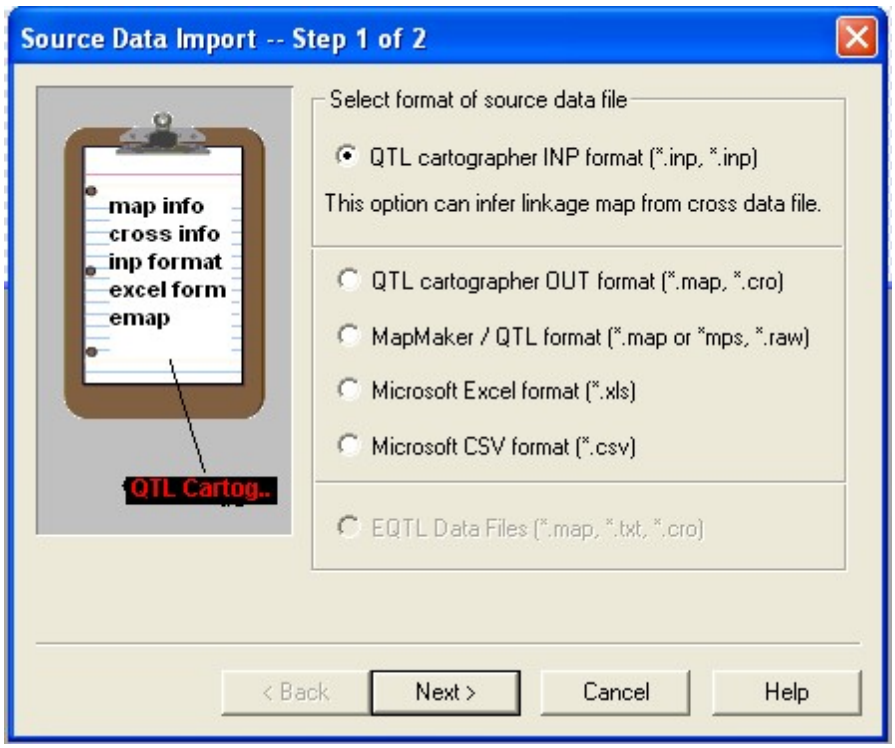
Application	Formats supported
MapMaker/QTL	.MAP – Map file .MPS – Map file .RAW – Cross data file
QTL Cartographer	.INP – Map and Cross data files

	.MAP – Map file .CRO – Cross data file
Microsoft Excel	.XLS .CSV

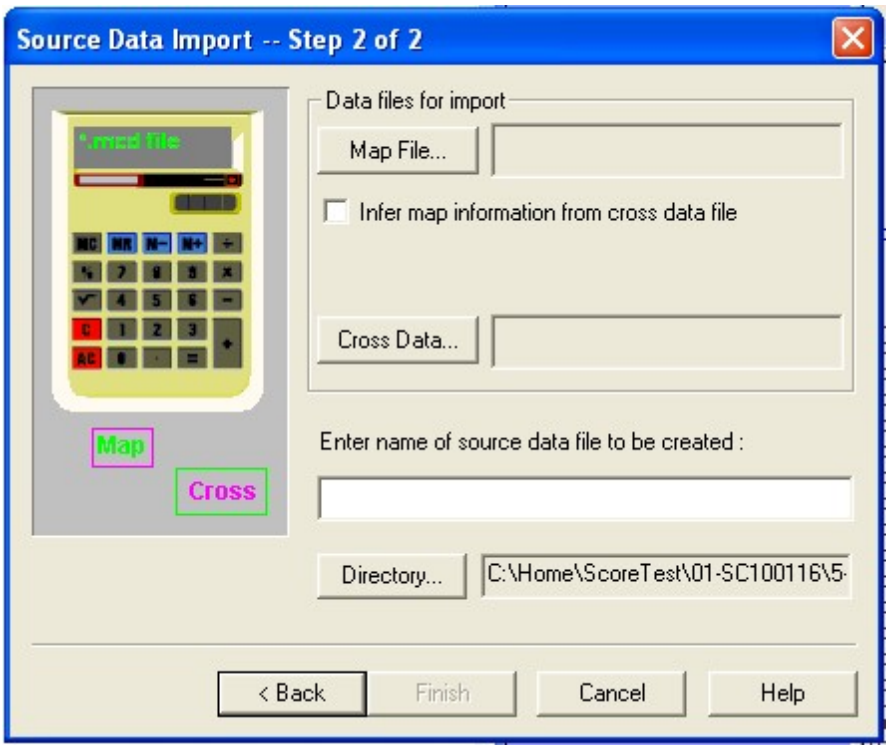
Note *If WinQTLCart displays an error message saying the file format is invalid or can't be recognized, then the extension may be wrong or the file's formatting renders it unusable in WinQTLCart. Open the file in Notepad and compare it to one of the sample files in the WinQTLCart directory.*

Importing source data files

1. Select File>Import. The Source Data Import dialog appears.



2. Select the import option you want. The first extension listed for each option is for the Map file, the second extension for the Cross Data file. After clicking Next, the second import dialog appears.

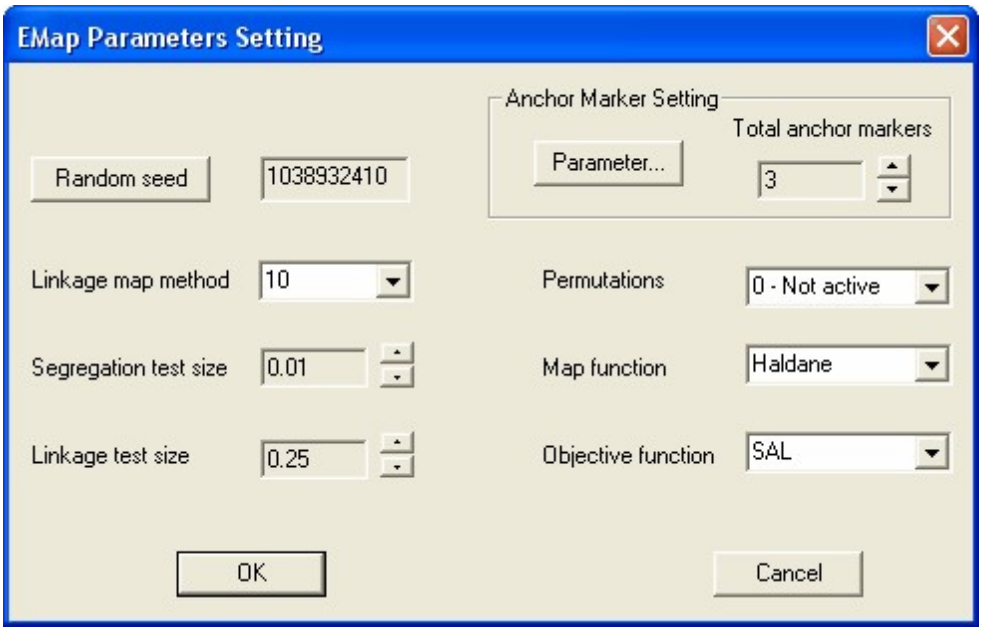


3. For the MapMaker/QTL and QTL Cartographer options, you need to locate and select the Map and Cross Data files by clicking the buttons. For Excel spreadsheets, you only need to specify one spreadsheet (WinQTLCart disables the Cross Data button for Excel imports).

4. By check "Infer map information from cross data file", WinQTLCart will infer map information from cross data file by using Emap function.

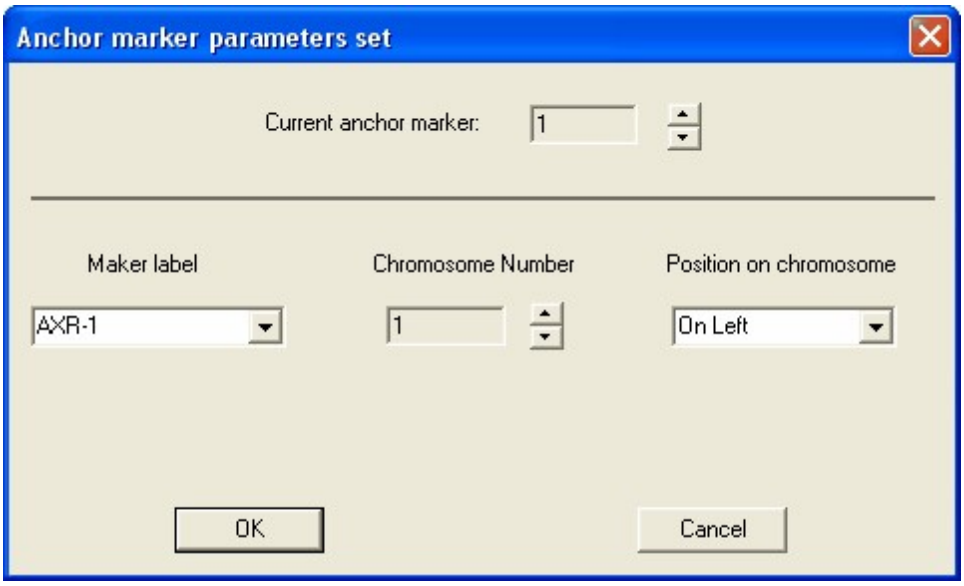
5. Enter a file name for the source data file that WinQTLCart will create. You don't need to specify an extension—WinQTLCart will take care of that.

6. Click the Finish button.



7. Emap parameters setting dialog will pop-out if you use Emap function to infer map information.
   - Click the button to change random seed for Emap function.
   - Linkage map method can take value 10, 11, 12, or 13.
   - Segregation test size can be 0.01 to 0.20.
   - Linkage test size can be 0.15 to 0.49.
   - Now, permutaion function is not active.
   - Map function can be Haldane or Kosambi.
   - Objuective function can be SAL or SAR.

8. Set anchor markers by using control group of Anchor marker setting .
   - Click to select anchor marker number.
   - Click button parameter to open window of Anchor marker parameters set
   - Select marker label, chromosome and position on chromosome for each marker (Current anchor marker).
   - Click button OK to finish parameters set.



Note:

- If the import was successful, you'll see a dialog saying the files were successfully imported and the source data file has been saved.
- If the import didn't work, the reason is likely that the files' formatting does not conform to a standard WinQTLCart expects. To troubleshoot 86 this problem, open one of WinQTLCart's sample files in Notepad and compare it to the file you specified. (The topic MCD file format 39 in this manual also describes the file format.) Correct any formatting problems in your specified file and try importing again. If you're still unsuccessful, please contact WinQTLCart tech support 86.
- More detail help for Emap function, see QTL Cartograhper's manul.

Notes

- WinQTLCart includes sample source data files for import. Run some tests using these files or open them up to see the kind of data formatting WinQTLCart expects to see.
- For Excel worksheets, WinQTLCart expects to see the following worksheet names in the file: BasDat, ChrDat, and CroDat. WinQTLCart includes a sample file, NewMcd.xls, that demonstrates the formatting it expects to see. (If you want, you can make a copy of NewMcd.xls and modify it for your data.)
- For INP format, one cross data file is needed if you use Emap to infer map information.

Related topics

Compatible programs and formats 1

Exporting source data and results

The following table summarizes WinQTLCart's export options:

Export ...	...from the...	...to...	...in these formats...
Source data	Main window	QTL Cartographer 30	.INP – Map and cross data files
Source data	Main window	QTL Cartographer 30	.MAP – Map file .CRO – Cross data file

Source data (minus individuals with certain OTrait values)	Main window	WinQTLCart 30	.MCD (with option to delete individuals with a certain OTrait value)
Source data (one or more chromosome(s) with reverse marker position)	Main window	WinQTLCart 30	.MCD (with reverse marker position in some chromosomes)
Source data (with certain selected traits)	Main window	WinQTLCart 30	.MCD (with some selected traits)
Source data	Main window	Microsoft Excel 31	.XLS
Results	Graph window	Microsoft Excel 31	.XLS
Results	Graph window	Text	.TXT (tab-delimited)

Notes *Files are exported to the current working directory 25.*

Exporting source data to QTL Cartographer

1. With the source data displayed in the Main window's Data pane, select File>Export.
2. In the Export Source Data dialog, select one of the following options:

Select this…	**…to output these files**
QTL Cartographer INP format	.INP files for both the map and cross data
QTL Cartographer OUT format	.MAP for the map file .CRO for the dross data file

3. Edit the Map or Cross filenames, as needed.
4. Click OK. WinQTLCart exports the files to the current working directory.

Exporting source data to an MCD file

Although WinQTLCart saves source data to its own .MCD format, you can use the export dialog to strip an .MCD file of individuals with OTrait values. You would do this when you want to analyze the data separate from the traits.

A. MCD file (delete individuals with a certain OTrait value)

1. With the source data displayed in the Main window's Data pane, select File>Export
2. In the Export Source Data dialog, select MCD file (delete individuals with a certain OTrait value).
3. Edit the source file name, as needed. The file will be saved to the current working directory 25.
4. From the OTrait Value pull-down menu, select the trait to be stripped from the MCD file.
5. Click OK. WinQTLCart exports the file to the current working directory 25.

B. MCD file (reverse one or several chromosomes)

1. With the source data displayed in the Main window's Data pane, select File>Export
2. In the Export Source Data dialog, select MCD file (reverse one or several chromosomes).
3. Edit the source file name, as needed. The file will be saved to the current working directory 25.
4. From the Chrom. Number Edit Box, type in chromosome numbers separated by comma.
5. Click OK. WinQTLCart exports the file to the current working directory 25.

C. MCD file (only selected traits are included)

1. With the source data displayed in the Main window's Data pane, select File>Export
2. In the Export Source Data dialog, select MCD file (only selected traits are included).
3. Edit the source file name, as needed. The file will be saved to the current working directory 25.
4. From the Trait Number Edit Box, type in trait numbers separated by comma or hyphen.
5. Click OK. WinQTLCart exports the file to the current working directory 25.

Exporting results from the Graph window

The Graph window doesn't export results, per se (there's no Export menu), but you can save the results in these formats:

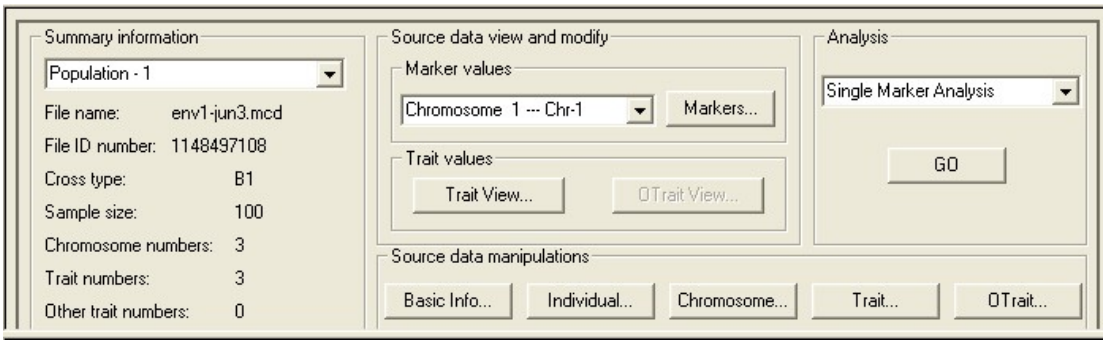
- WinQTLCart mapping result file (.QRT).
- Excel file (.XLS), LR/LOD values to a worksheet labeled "LR", QTL information to a worksheet labeled "QTLs", and points obtained through graph trace function to a worksheet labeled "Points".
- Text file (.TXT), in a tab-delimited format.

Simply select the appropriate command from the Graph window's File menu, specify the directory and filename in the Save As dialog, and click OK. WinQTLCart will display a confirmation dialog that the file has been created.

Working with source data files

WinQTLCart's source data files have a .MCD extension. A .MCD file is a text file that adheres to a specific format 39 that includes all the information WinQTLCart needs for QTL mapping analysis.

When you open a source data file 32, WinQTLCart verifies the file's formatting and displays its basic information in the Main window's Form pane.



Note *Although you can open other text and result files in WinQTLCart, only .MCD file information is displayed in the Form pane. WinQTLCart will continue showing the last .MCD file you viewed in the Form pane, even if you open or switch to text or result files.*

The Summary Information box tells you the basics on the selected source data file. From here, you can go ahead and select an analysis method, if you wish.

However, the form's buttons and pull-down lists let you look at the source data file in more detail and also perform the following actions:

- View information of markers, traits and other traits (also called categorical traits) in a formatted way.
- Add/edit/delete marker genotype data 32 for each chromosome in the file, including adding or

deleting individuals
- Add/edit/delete trait values 33 and other traits
- Edit map 34 and cross 35 information
- Add new experimental data (individuals)
- Try out different data configurations, such as retaining certain individuals' chromosomes

You may find that you need to add, edit, or delete data due to import errors. Some errors can be caused by mistaking cross data for map data, and vice versa.

WinQTLCart's tools for editing marker, trait, and other information offer a safer and more organized approach than if you were to do the same thing by hand in a text editor. If you need to alter the source data's information in any way, it is highly recommended you use WinQTLCart.

Opening source data files

There are several ways to open a prepared source data file from the Main window.
- Select File>Open
- Click the Open button on the toolbar
- Use the keyboard command Ctrl+O
- Double-click or right-click on the Source Files node in the Tree pane 10

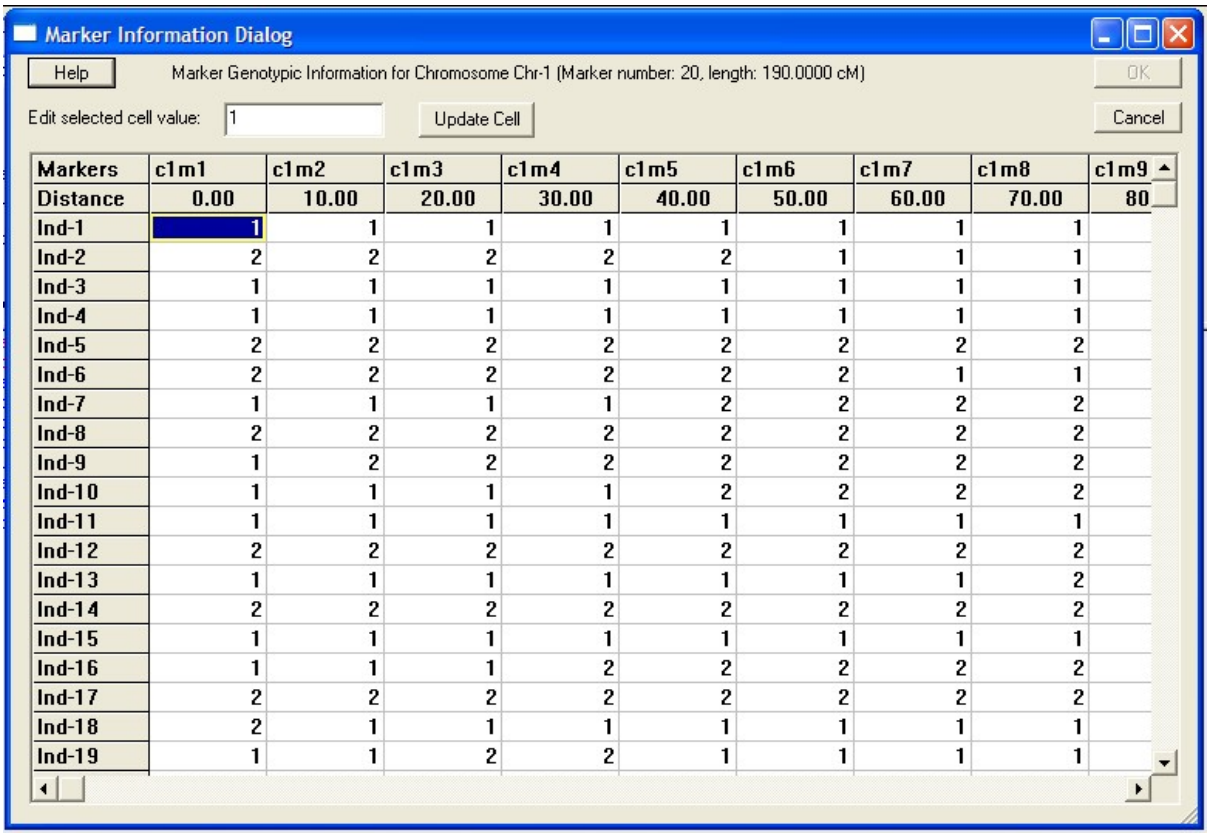
Any of these methods opens the standard Windows Open dialog box. The dialog defaults to the current working directory 25. If you open an .MCD file from a different directory, WinQTLCart will save the mapping result files to that location.

Also you can double click a .MCD file in Windows File Explorer to open the source data file automatically.

Working with a source file's marker genotype data

At the Source data view and modify pane on the Main window, select the chromosome you want to work with from the pull-down list and click Markers button to open the Marker Information dialog.

At the Marker Information dialog, Marker Labels are the column headings, Individuals are the rows. You can enlarge the dialog by dragging its border toward or away from the center of the screen. (The pointer is a two-headed arrow when it is in the correct position.)



Markers	c1m1	c1m2	c1m3	c1m4	c1m5	c1m6	c1m7	c1m8	c1m9
Distance	0.00	10.00	20.00	30.00	40.00	50.00	60.00	70.00	80
Ind-1	1	1	1	1	1	1	1	1	
Ind-2	2	2	2	2	2	1	1	1	
Ind-3	1	1	1	1	1	1	1	1	
Ind-4	1	1	1	1	1	1	1	1	
Ind-5	2	2	2	2	2	2	2	2	
Ind-6	2	2	2	2	2	2	1	1	
Ind-7	1	1	1	1	2	2	2	2	
Ind-8	2	2	2	2	2	2	2	2	
Ind-9	1	2	2	2	2	2	2	2	
Ind-10	1	1	1	1	2	2	2	2	
Ind-11	1	1	1	1	1	1	1	1	
Ind-12	2	2	2	2	2	2	2	2	
Ind-13	1	1	1	1	1	1	1	2	
Ind-14	2	2	2	2	2	2	2	2	
Ind-15	1	1	1	1	1	1	1	1	
Ind-16	1	1	1	2	2	2	2	2	
Ind-17	2	2	2	2	2	2	2	2	
Ind-18	2	1	1	1	1	1	1	1	
Ind-19	1	1	2	2	1	1	1	1	

Changing a cell's contents

For modifying a cell's value, click in the cell and type in a new value. (Or, click in a cell and enter the value in the Edit selected cell value field.)

You can change as many cells as you like. Click Update Cell to save your changes as you go. you can click OK to save all changes and close the dialog.

Click Cancel to close the dialog without saving any of your changes (including any changes saved with the Update Cell button).

Working with a source file's traits values

Traits: At the Trait values pane on the Main window, click the Trait View button to open the Trait Information dialog.

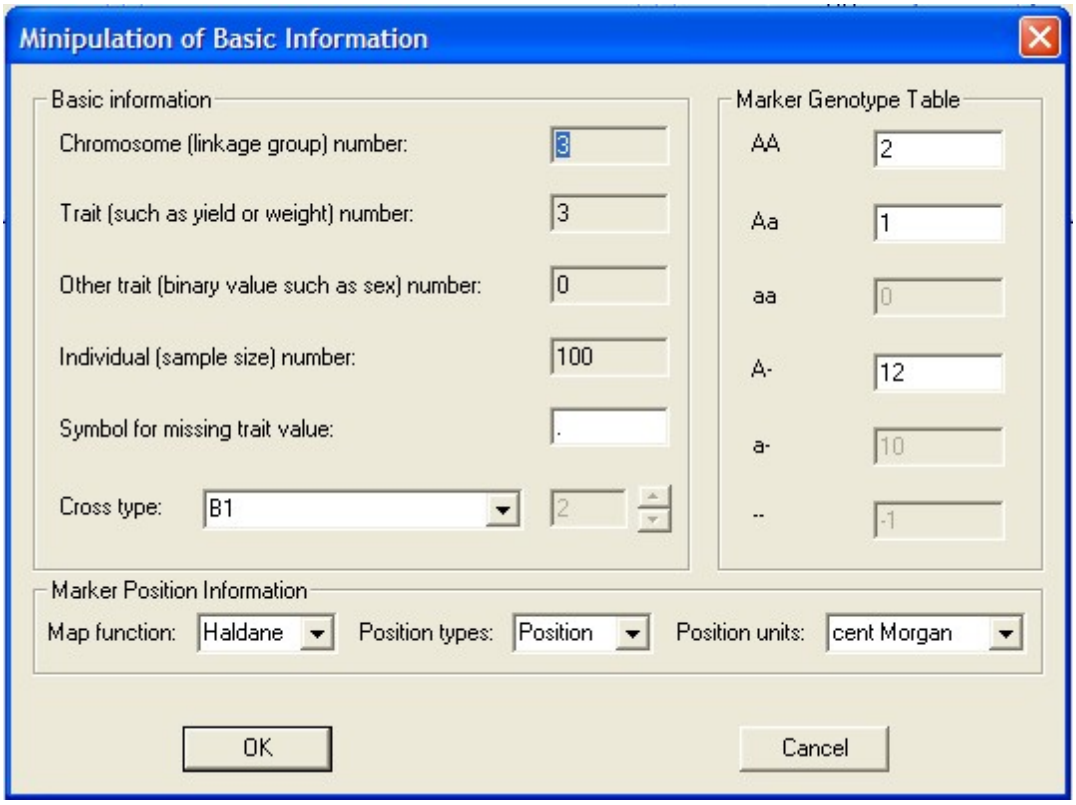
Other Traits: If the source data file contains other traits, then click the OTrait View button to open the Other Trait Information Dialog.

For more information on working with this dialog, and on modifying values, see the following topics:

Working with a source file's marker genotype data 32

Working with a source file's basic information

At the Source data manipulations pane on the Main window, click the Basic Info button to open the Manipulation of Basic Information dialog.



Symbol for missing trait value. Enter a symbol to use for missing traits value, based on your trait data.

Marker translation table. Edit or add symbols you want to use for these genotype markers; you can enter any alphanumeric character(s) as a symbol. These translations apply to the experimental design you selected at the left of the dialog.

Note *WinQTLCart assumes that the A allele is diagnostic for the High (parental 1) line and the a allele is diagnostic for the Low (parental 2) line. A minus sign (-) means the allele is unknown. WinQTLCart uses the numbers (2, 1, 0, 12, 10, -1) to determine how to encode the output of the genotypes. The alphanumeric tokens you enter here indicate how you have coded the markers in the source data.*

Cross Information

Select the experimental design option based on your data. You can select only one option.

- **B1.** Backcross, with 1=parental line to which the F1 line was crossed.
- **B2.** Backcross, with 2=parental line to which the F1 line was crossed.
- **Ri0.** Recombinant inbred line, derived by doubled haploid lines.
- **Ri1.** Recombinant inbred line, derived by selfing.
- **Ri2.** Recombinant inbred line, derived by sib mating.
- **SF.** Selfed intercross line. Enter an integer indicating the generation. Limit of 2.
- **RF.** Randomly mated intercross line. Enter an integer indicating the generation.

T(B1)SF. Test cross, with genotyping done on an intercross (SF) and phenotyping on a cross (B1) derived from that intercross. Enter an integer indicating the generation.
T(B1)RF. Test cross, with genotyping done on an intercross (RF) and phenotyping on a cross (B1) derived from that intercross. Enter an integer indicating the generation.
T(B2)SF. Test cross, with genotyping done on an intercross (SF) and phenotyping on a cross (B2) derived from that intercross. Enter an integer indicating the generation.
T(B2)RF. Test cross, with genotyping done on an intercross (RF) and phenotyping on a cross (B2) derived from that intercross. Enter an integer indicating the generation.

Map Function
A map function is a mathematical relationship between recombination probabilities and map distances. Select the function you want, based on the interference to be assumed.

- **Haldane**. The default option. Assumes no crossover interference.
- **Kosambi**. Assumes some interference.
- **Fixed**. The Morgan mapping function. Assumes complete interference.

Note *These mapping functions, among others, are discussed at length in Ben Lui's book,* Statistical Genomics: Linkage, Mapping and QTL Analysis *(1998).*

Distance type

- **Position**. Indicates that the numbers indicate positions from the left telomere of the current chromosome. This means the numbers should be in ***increasing*** order.
- **Interval**. Indicates that the numbers are for the interval distance after a marker. This means that ***the last number, and the last number only***, should be ***zero***.

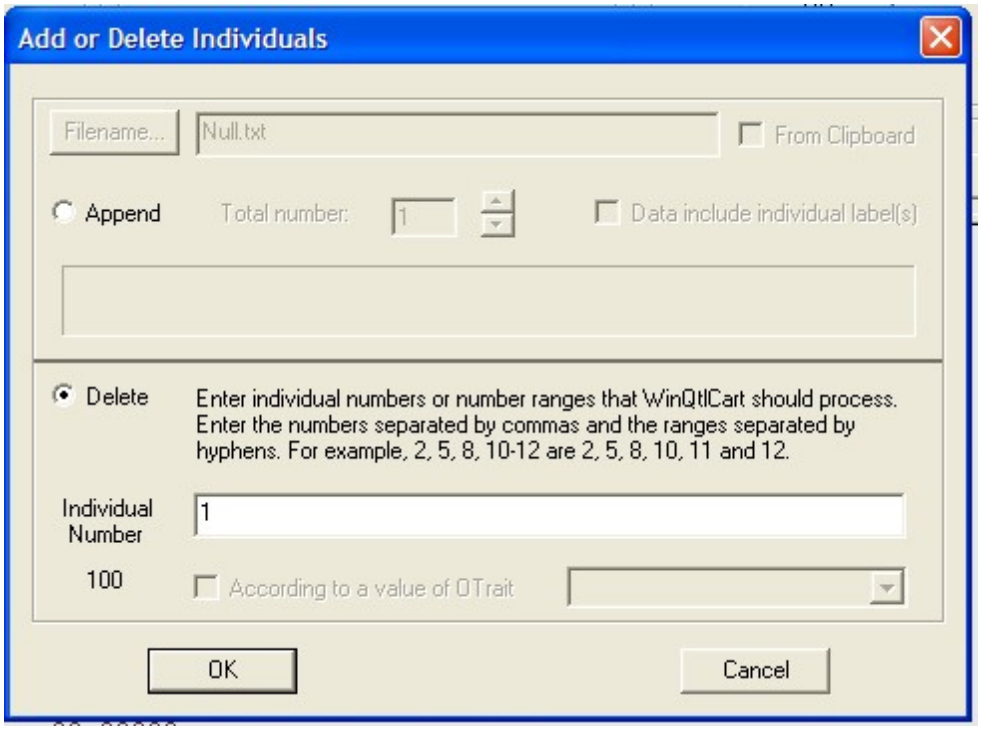
Distance units

- **centiMorgan (cM)**. Very small. 100 centiMorgans=1 Morgan.
- **Morgan**. The distance over which, on average, one crossover occurs per meiosis.
- **Recombination**. Percentage of crossover events that occur between two markers.

Related Topics
MCD file format 39

Working with source file's individual information

At the Source data manipulations pane on the Main window, click the Individual button to open the Add or Delete Individuals dialog.



Append and Delete. Click Append radio button to add individual(s) into active source data and click Delete radio button to delete one or more individual from active source data.

1. Append individual(s)

WinQTLCart can read the individual information through a properly formatted text file (data separated by spaces, not commas) and click Filename button to indicate the filename. (Use the Notepad button to call up an empty text file if you need to do impromptu editing.) To check From Clipboard button if the individual information is already in Windows clipboard. Please check the Data include individual label(s) button if the individual data include individual label. Click OK button to append the individual(s) after set the correct Total (individual) number.

There are two ways to read data.

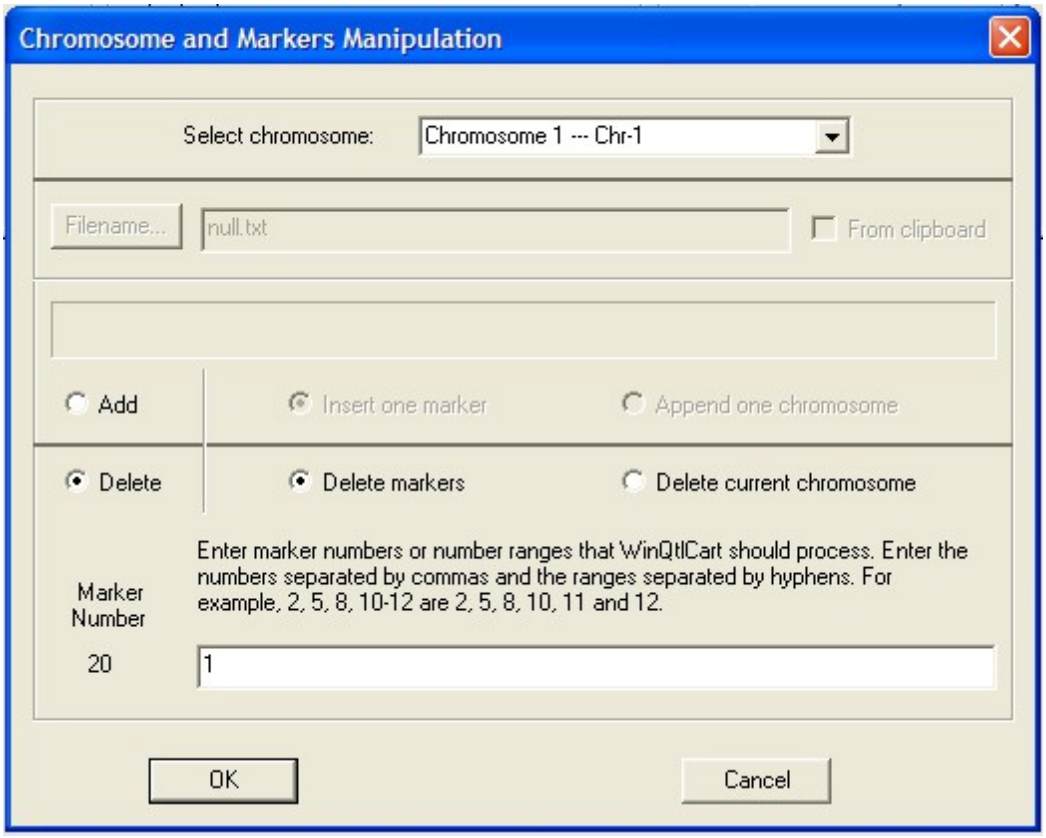
If your data is...	Then click...	Comments
In a text file	**Filename…** to display the Open dialog box.	In the Open dialog, select the text file that has the values you want to add and click OK.
On the Windows clipboard	**Data from clipboard** check it to transfer the data to the edit window.	You should select and copy the data in other text editor such as Windows notepad.

2. Delete individual(s)

In the Individual Number text edit box, enter the individual(s) you want to delete, separated by commas or hyphen. Click OK button to finish.

Working with source file's chromosome information

At the Source data manipulations pane on the Main window, click the Chromosome button to open the Chromosome and Markers Manipulation dialog.



Add and Delete. Click Add radio button to add a chromosome or a marker into active source data and click Delete radio button to delete current chromosome or marker(s) in current chromosome from active source data.

Select chromosome. Select one chromosome from the pull-down menu as the current chromosome.

1. Insert one marker in current chromosome

WinQTLCart can read the marker information through a properly formatted text file and click Filename button to indicate the filename. To check From Clipboard button if the marker information is already in Windows clipboard.

2. Insert one chromosome

WinQTLCart can read the chromosome information through a properly formatted text file and click Filename button to indicate the filename. To check From Clipboard button if the chromosome information is already in Windows clipboard.

3. Delete current chromosome

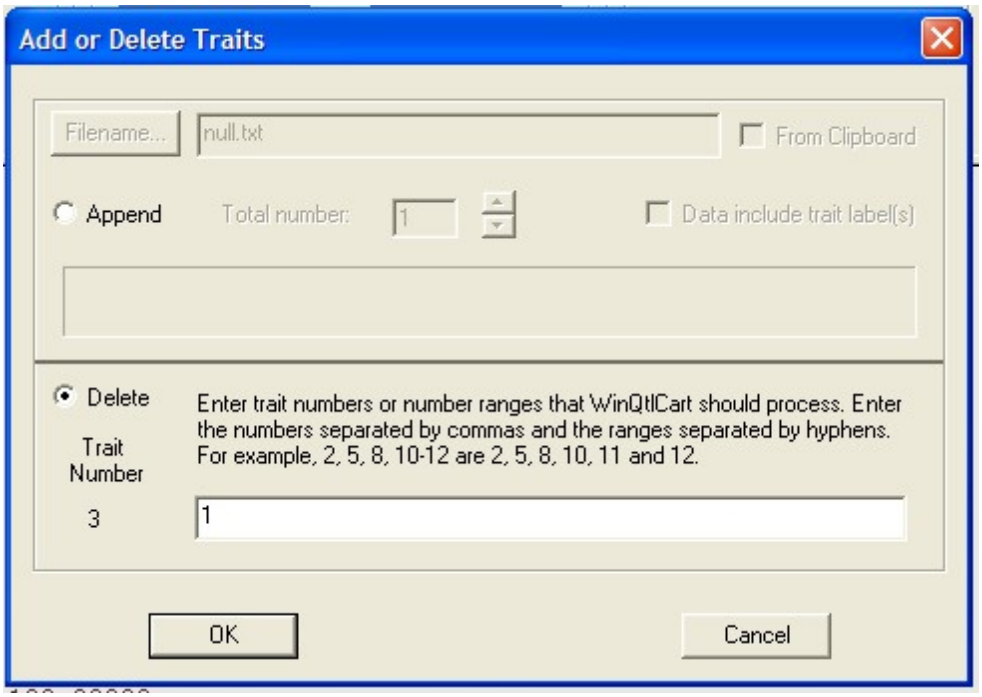
Click Delete current chromosome radio button and click OK button.

4. Delete marker(s) in current chromosome

Click Delete markers radio button and in the Marker Number text edit box, enter the marker(s) you want to delete, separated by commas or hyphen.

Working with source file's trait information

At the Source data manipulations pane on the Main window, click the Trait button to open the Add or Delete Traits dialog.



Add and Append. Click Append radio button to append trait(s) into active source data and click Delete radio button to delete trait(s) from active source data.

1. Append trait(s)

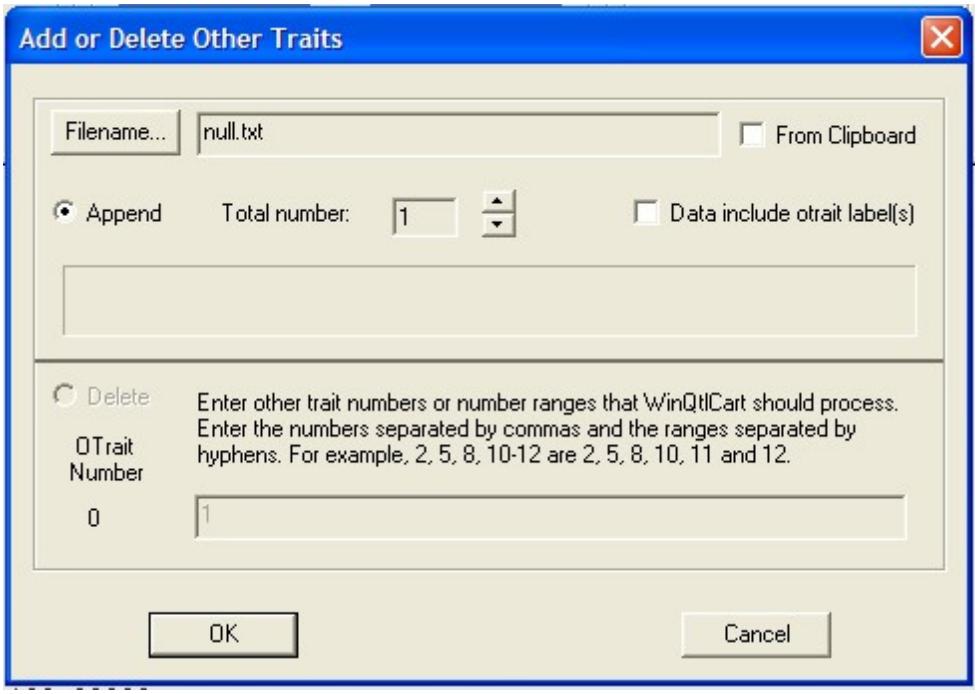
WinQTLCart can read the trait information through a properly formatted text file and click Filename button to indicate the filename. To check From Clipboard button if the trait information is already in Windows clipboard. Please check the Data include trait label(s) button if the trait data include trait label. Click OK button to append the trait(s) after set the correct Total (trait) number.

2. Delete trait(s)

In the Trait Number text edit box, enter the Trait(s) you want to delete, separated by commas or hyphen.

Working with source file's other trait information

At the Source data manipulations pane on the Main window, click the OTrait button to open the Add or Delete Other Traits dialog.



Add and Append. Click Append radio button to append other trait(s) into active source data and click Delete radio button to delete other trait(s) from active source data.

1. Append other trait(s)

WinQTLCart can read the other trait information through a properly formatted text file and click Filename button to indicate the filename. To check From Clipboard button if the other trait information is already in Windows clipboard. Please check the Data include otrait label(s) button if the other trait data include label. Click OK button to append the other trait(s) after set the correct Total (other trait) number.

2. Delete other trait(s)

In the OTrait Number text edit box, enter the Other Trait(s) you want to delete, separated by commas or hyphen.

MCD file format

WinQTLCart .MCD source data files use tokens to indicate the meaning of data. This topic describes valid tokens used in .MCD source data files.

The line numbers in this topic refer to the sample .MCD file, "NewMcd.mcd," which is included as part of the WinQTLCart distribution.

Token #FileID (line 1)

File's ID number; usually a 10-digit number.

Token /* and */ (Lines 2 – 5)
Insert multiple-line comments between these tokens.

Token #bychromosome (Line 6)
Indicates start of chromosome information.

Token // (Lines 6 – 10)
Insert one-line comments after the double-slash.

Token –type (Line 7)
Indicates how marker positions are numbered along the chromosome. It takes one parameter that can be either "position" or "interval".

- **Position** indicates that the numbers are positions from the left telomere of the current chromosome. So numbers should be in ***increasing*** order.
- **Interval** indicates that the numbers are the interval distance after a marker. So ***the last number and only the last number*** in the series should be ***zero***.

```
-type

position  0.0  9.3  17.2  29.9  38.7  52.8  57.8  72.4  76.6  93.2  97.0  115.5
116.5

interval  9.3  7.9  12.7  8.8  14.1  5.0  14.6  4.2  16.6  3.8  18.5  1.0  0.0
```

Token –function (Line 8)
Indicates which map function is used to transfer recombination frequency (r) between markers to distance in Morgan (M). The parameter can be an integer from 1 – 8. Haldane and Kosambi are the two most useful map functions.

Code	Reference	Note
1	Haldane (1919)	Default
2	Kosambi (1944)	
3	Morgan (1994)	"Fixed"
4	Carter and Falconer (1951)	
5	Rao et al. (1979)	0 £ p £ 1
6	Sturt (1976)	L
7	Felsenstein (1979)	-¥ < K < ¥, K ¹ 2
8	Karlin (1984)	Binomial, N > 0

The following Haldane and Kosambi formula can be used to convert marker distance from r to M or vice versa.

Haldane:	dM = -0.5ln(1-2r)	r = 0.5(1-exp(-2dM)
Kosambi:	dM = 0.25ln((1+2r)/(1-2r))	r = (1-exp(-4dM))/(2(1+exp(-4dM)))

Token –Units (Line 9)
Indicates unit of marker positions. There are three choices:

- cM (centiMorgan)
- M (Morgan)

- r (Recombination frequency) - If you choose this parameter, then token -function should be 3 (Morgan)

Token –chromosome (Line 10)
Indicates total number of chromosomes for source data.

Token –maximum (Line 11)
Indicates the maximum number of markers for a chromosome for all of the source data.

Token –named (Line 12)
Either **yes** or **no**.
- Yes means markers have names
- No means markers will not have names.

Token –start and –end (Line 13 and Line 55)
Use these tokens to start and end the marker position data of all chromosomes.

Token –Chromosome (Line 14, 28, and 41)
Indicates chromosome name. The marker position data for this chromosome will start with the next line.

Token #bycross (Line 56)
Indicates that cross information is to begin.

Token –SampleSize (Line 57)
Indicates sample size or individual number.

Token –Cross (Line 58)
Indicates codes for crosstype mating design (see the following table).

Code	Design	Examples
Bi	Backcross to Pi	B1, B2
Bij	Backcross j times to Pi	B13, B25
SFi	Selfed generation i intercross	SF2, SF6
RFi	Randomly mated generation i intercross	RF2, RF3
RI0	Doubled haploid	RI0
RI1	Recombinant inbred via selfing	RI1
RI2	Recombinant inbred via sib mating	RI2
T(Bi)SFj	Testcross of SFi to Pj	T(B1)SF3
T(SFi+j)SFi	Testcross of SFi for j generations	T(SF4)SF3
T(Bj)RFi	Testcross of RFi to Pj	T(B1)RF3
T(D3)SFi	Design III	T(D3)SF5

Token –traits (Line 59)
Indicates trait number of source data.

Token –otraits (Line 60)
Indicates other trait number of source data. Other trait (also called a categorical trait) is the trait with qualitative or categorical values, such as sex; color, and so on. Other traits can be used as factors that can be "regressed out" in regression analysis. This means a regression of the quantitative trait of interest on the categorical trait will have been performed and the residuals used as the phenotypes in the analysis.

Token –missingtrait (Line 61)
Indicates the symbol for missing trait value.

Token –case (Line 62)
Either **yes** or **no**.

- Yes means all comparisons are case dependent.
- No means all names of individuals, markers and traits are converted to lower case to make comparisons.

Token –TranslationTable (Line 62)
The token with the table (next 6 lines of data) will define how marker genotype data are translated. There are six rows and three columns (18 positions) in the table. Here is the default translation table:

```
-TranslationTable
AA   2   2
Aa   1   1
aa   0   0
A-  12  12
a-  10  10
--  -1  -1
```

- The first column is the genotype. The program assumes that the A allele is diagnostic for the High (parental 1) line and the a allele is diagnostic for the Low (parental 2) line. A minus sign (-) means the allele is unknown (missing). Dominant as well as co-dominant markers can be encoded.
- The middle column is how the output of these genotypes will be encoded.
- The third column is how you will code the marker genotype data in this source data file. Just about any set of tokens can be used for the third column (corresponding to your dataset), but DO NOT change the first two columns.

The above TranslationTable maps 2 to 2, 1 to 1, 0 to 0, etc. Just about any set of tokens can be used for the third column, but DO NOT change the first two columns. If you encoded your P1 homozygotes as BB, heterozygotes as Bb, etc, your translation table might appear as:

```
-TranslationTable
AA   2  BB
Aa   1  Bb
aa   0  bb
A-  12  B
a-  10  b-
--  -1  --
```

Anything in the data file that is not recognized (doesn't match something in column 3) will become unknown (-1) in the output.

Important: You need ***all*** 18 tokens following the -TranslationTable token and the first two columns can't be altered. You should only alter the last column.

Token –start markers and –stop markers (Line 70 and Line 109)
Use these tokens to start and end the marker genotype data of all chromosomes. Please keep the same chromosome and marker order as the data between **token –start** and **token -end** above.

You can organize the data by marker or individual:

- Order by marker: For each marker, you provide the genotype data for all individuals. The order of the

individuals must be the same for each marker.

- Order by individuals: For each individual, you provide the genotype data for all markers (all chromosomes). The order of chromosomes and markers has to be the same.

```
-start individuals markers
Ind1  2  2  1  1  2  2  2  2  2
Ind2  2  2  2  1  2  1  2  2  1
Ind3  2  2  2  2  2  1  2  1  1
Ind4  2  1  2  2  2  2  1  1  1
Ind5  2  1  2  2  2  2  1  1  1
-stop individuals markers
```

Note that the tokens are different and the first column is the individual's label.

Token –start traits and –stop traits (Line 110 and Line 114)
Use these tokens to start and end the trait values. The data should be organized by the trait's order for the trait value. That is, for each trait, you give the trait value of all individuals. If organized by the individual, then for each individual, you provide the trait value of all traits.

```
-start individuals traits 2 Trait_1 Trait_2 named
Ind1      5.0    15.0
Ind2      5.3    15.3
Ind3      6.2    16.2
Ind4      4.1    24.1
Ind5      5.5    25.5
-stop individuals traits
```

Note that the tokens are different and there are trait number and trait labels after the token -start individuals traits. The label "named" means data has trait names.

Token –start otraits and –stop otraits (Line 115 and Line 118)
Use these tokens to start and end the other trait values. The data must be organized by the other trait's order; that is, for each other trait, you give the other trait value of all individuals. If you order by individuals, then for each individual, you give the other trait value of all other traits.

Example:

```
-start individuals otraits 2 sex brood named
Ind1      M      1
Ind2      F      1
Ind3      M      0
Ind4      M      1
Ind5      M      1
-stop individuals otraits
```

Please notice that the tokens are different and there are other trait numbers and other trait labels after the token -start individuals otraits. Label "named" means data has other trait names.

Token –quit and –end (Line 119 and Line 120)
Indicate the end of the source data file.

Token -population
Indicate the population number and the rest of .MCD filename.
Please notice that you should build one .MCD file for each population.

Example:

-population 4 env2-jun3.mcd env3-jun3.mcd env4-jun3.mcd

There are totally 4 populations and the 4 .MCD files include current file, and file "env2-jun3.mcd", file "env3-jun3.mcd" and file "env4-jun3.mcd". All .MCD files should be in same directory.

Token -xchromosome
Indicate the X chromosome number and cross type that will not be the same as rest of chromosomes.

Creating a new source data file from raw data

WinQTLCart can help you convert your raw source data (including map and cross information) into formatted MCD files. WinQTLCart can run analysis methods only on MCD files.

The goal of this process is to create a file that looks like the MCD data file (described in the MCD data file format 39 topic). Although WinQTLCart can't automate this process for you, it does provide you with tools to make a clean transition from raw source data to rigorously formatted data file.

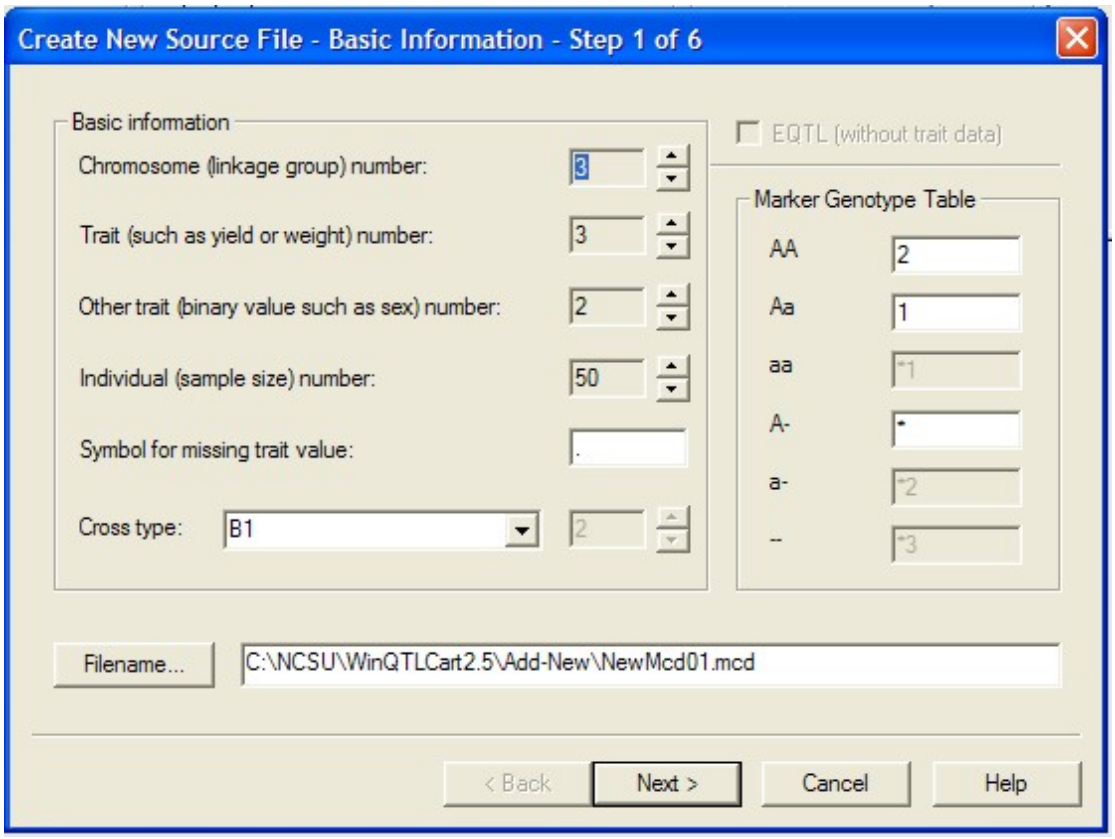
The easiest way to work with this function is to have individual, properly formatted text files for each data type (marker genotype, individuals, etc.). Individual files are quicker to select and import. Otherwise, you do have to do a lot of copy and paste.

Here's a checklist of files you should have on hand, along with sample files in the WinQTLCart2.5\Add-New directory you can use to create your own files.

- Chromosome labels and number. See 201-chlab-mknum.txt
- Marker labels. See 301-mklab.txt that shows one file for all chromosomes.
- Marker positions. See 302-mkpos.txt that shows one file for all chromosomes.
- Marker genotypes. One file for all chromosomes and there are 4 different cases:
  Case 1: Individual order with individual label, See 521-mkgen-ind-lab.txt
  Case 2: Individual order without individual label, See 521-mkgen-ind-nolab.txt
  Case 3: Marker order with marker label, See 541-mkgen-mk-lab.txt
  Case 4: Marker order without marker label, See 541-mkgen-mk-nolab.txt
- Trait values and labels. One file for all traits:
  Case 1: Individual order with trait label, See 522-tt-ind-lab.txt
  Case 2: Individual order without trait label, See 522-tt-ind-nolab.txt
  Case 3: Trait order with trait label, See 542-tt-tt-lab.txt
  Case 4: Trait order without trait label, See 542-tt-tt-nolab.txt
- Other trait values and labels. One file for all other traits:
  Case 1: Individual order with other trait label, See 523-ot-ind-lab.txt
  Case 2: Individual order without other trait label, See 523-ot-ind-nolab.txt
  Case 3: other trait order with other trait label, See 543-ot-ot-lab.txt
  Case 4: other trait order without other trait label, See 543-ot-ot-nolab.txt

An alternative to use separate files is to create a single file holding marker genotype, trait, and other trait information. But the data must be in individual order:
Case 1: has individual label, See 511-mkttot-lab.txt
Case 2: has not individual label See 511-mkttot-nolab.txt

1. Select File>New to open the Create New Source File – Step 1 of 6 dialog box.



Fill in the dialog based on your source data.

Basic information group box

Chromosome number. Enter the number of chromosomes in the source data.

Trait number. Enter the number of traits in the source data.

Other trait number. Enter the number of other traits in the source data. ("Other traits" are also known as categorical traits.)

Individual number. Enter the number of individuals in the source data.

Symbol for missing trait value. Enter a symbol to use for missing traits, based on your trait data.

Crossing type. Select the correct cross type for your data:

B1. Backcross, with 1=parental line to which the F1 line was crossed.

B2. Backcross, with 2=parental line to which the F1 line was crossed.

Ri0. Recombinant inbred line, derived by doubled haploid lines.

Ri1. Recombinant inbred line, derived by selfing.

Ri2. Recombinant inbred line, derived by sib mating.

SF. Selfed intercross line. Enter an integer indicating the generation. Limit of 2.

RF. Randomly mated intercross line. Enter an integer indicating the generation.

T(B1)SF. Test cross, with genotyping done on an intercross (SF) and phenotyping on a cross (B1) derived from that intercross. Enter an integer indicating the generation.

T(B1)RF. Test cross, with genotyping done on an intercross (RF) and phenotyping on a cross (B1) derived from that intercross. Enter an integer indicating the generation.

T(B2)SF. Test cross, with genotyping done on an intercross (SF) and phenotyping on a cross (B2) derived from that intercross. Enter an integer indicating the generation.

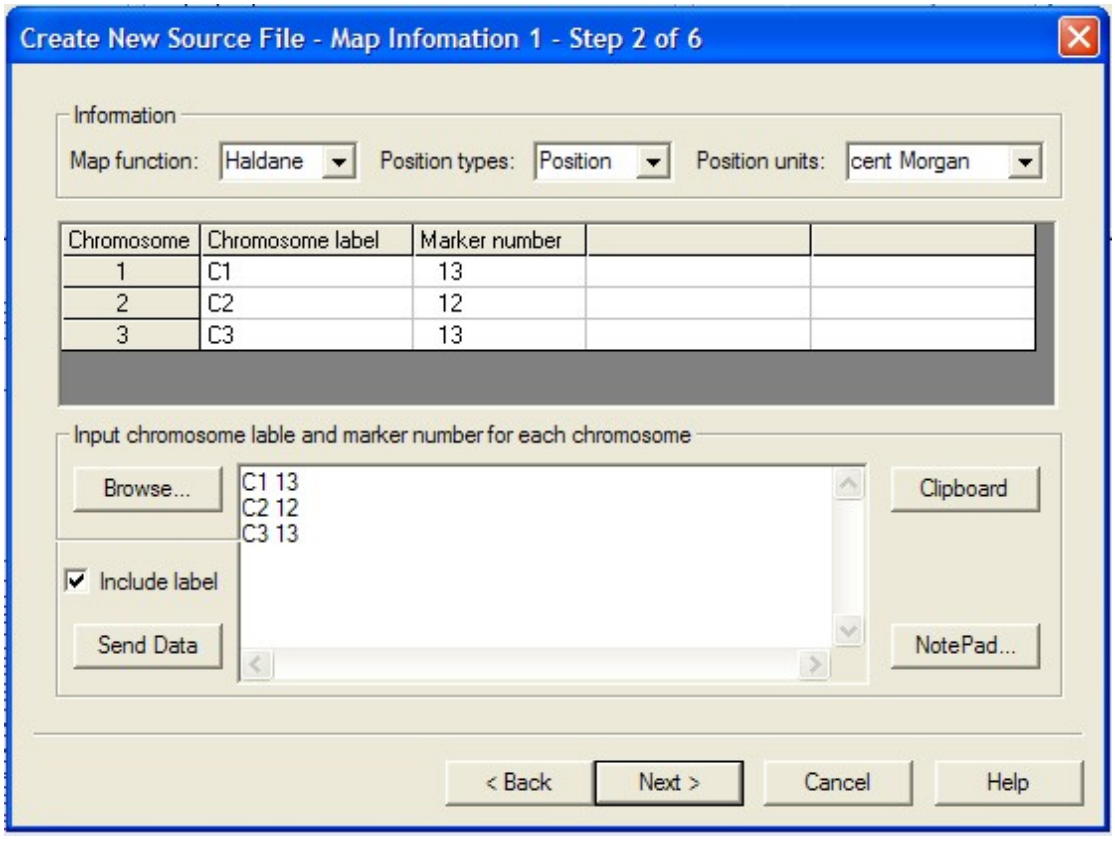
T(B2)RF. Test cross, with genotyping done on an intercross (RF) and phenotyping on a cross (B2) derived from that intercross. Enter an integer indicating the generation.

Marker genotype table. Edit or add tokens you want to use for these genotype markers; you can enter any alphanumeric character(s) as a token. These translations apply to the Crossing type you selected from the drop down. You must ensure that the chosen symbols in this translation table match those in your marker genotype data file.

Note *WinQTLCart assumes that the A allele is diagnostic for the High (parental 1) line and the a allele is diagnostic for the Low (parental 2) line. A minus sign (-) means the allele is unknown. WinQTLCart uses the numbers (2, 1, 0, 12, 10, -1) to determine how to encode the output of the genotypes. The alphanumeric tokens you enter here indicate how you have coded the markers in your source data.*

Source data file stem name. Enter a name for your source data file. As WinQTLCart creates new source data files, WinQTLCart will append extensions to this stem.

2. Click Next button to accept the values. The Create New Source File – Step 2 of 6 appears.



Fill in the dialog based on your source data.

Map function. A map function is a mathematical relationship between recombination probabilities and map distances. Select the function you want, based on the interference to be assumed.

- Haldane. The default option. Assumes no crossover interference.
- Kosambi. Assumes some interference.
- Fixed. The Morgan mapping function. Assumes complete interference.

Position type

- Position. Indicates that the numbers indicate positions from the left telomere of the current chromosome. This means the numbers should be in ***increasing*** order.
- Interval. Indicates that the numbers are for the interval distance after a marker. This means that ***the last number, and the last number only***, should be ***zero***.

Position units

- centiMorgans (cM). Very small. 100 centiMorgans=1 Morgan.
- Morgan. The distance over which, on average, one crossover occurs per meiosis.
- Recombination. Percentage of crossover events that occur between two markers.

Data-window. All the numbers you see here are place holders, waiting for you to input the real data.

Edit window. Data from your raw data file (or in Windows clipboard) is copied to this window first. You can edit data in this window.

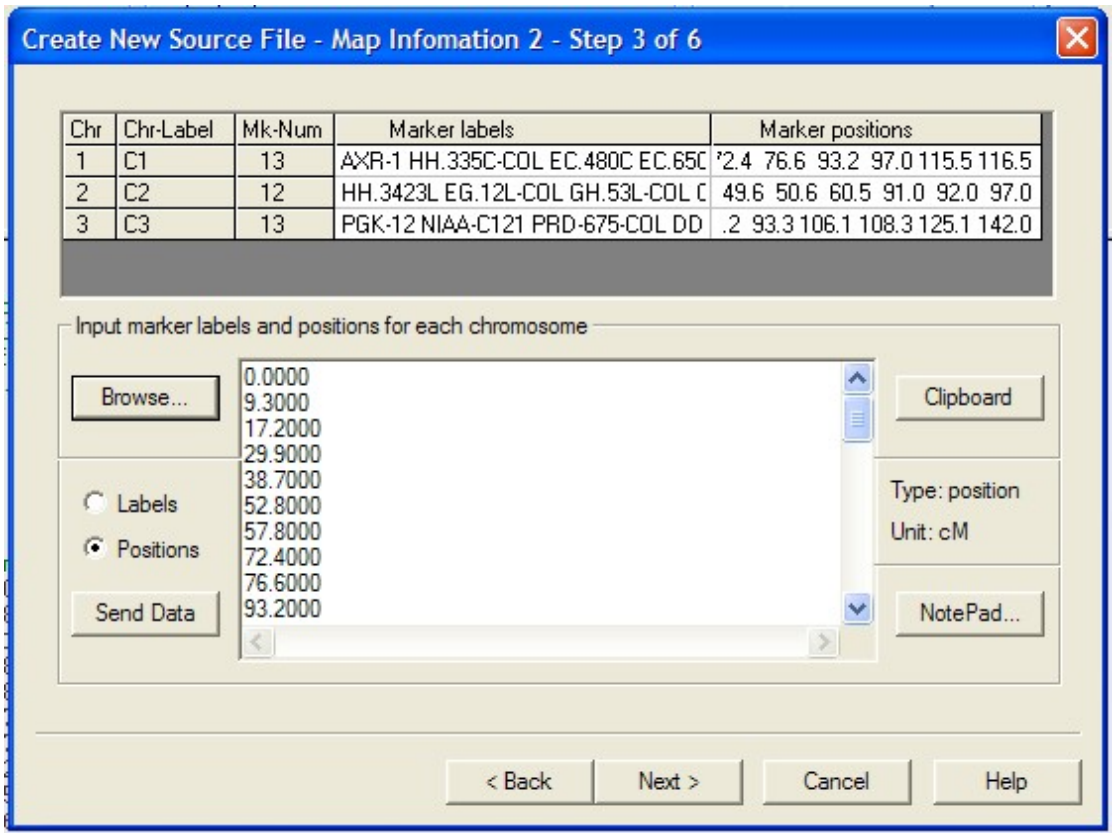
Browse Displays Windows Open dialog so you can select the data file. Selecting the file copies its contents to the Edit window.

Clipboard. Paste contents of Windows clipboard to Edit window.

Send Data. Check Include label if necessary, then click Send Data to update the data-window.

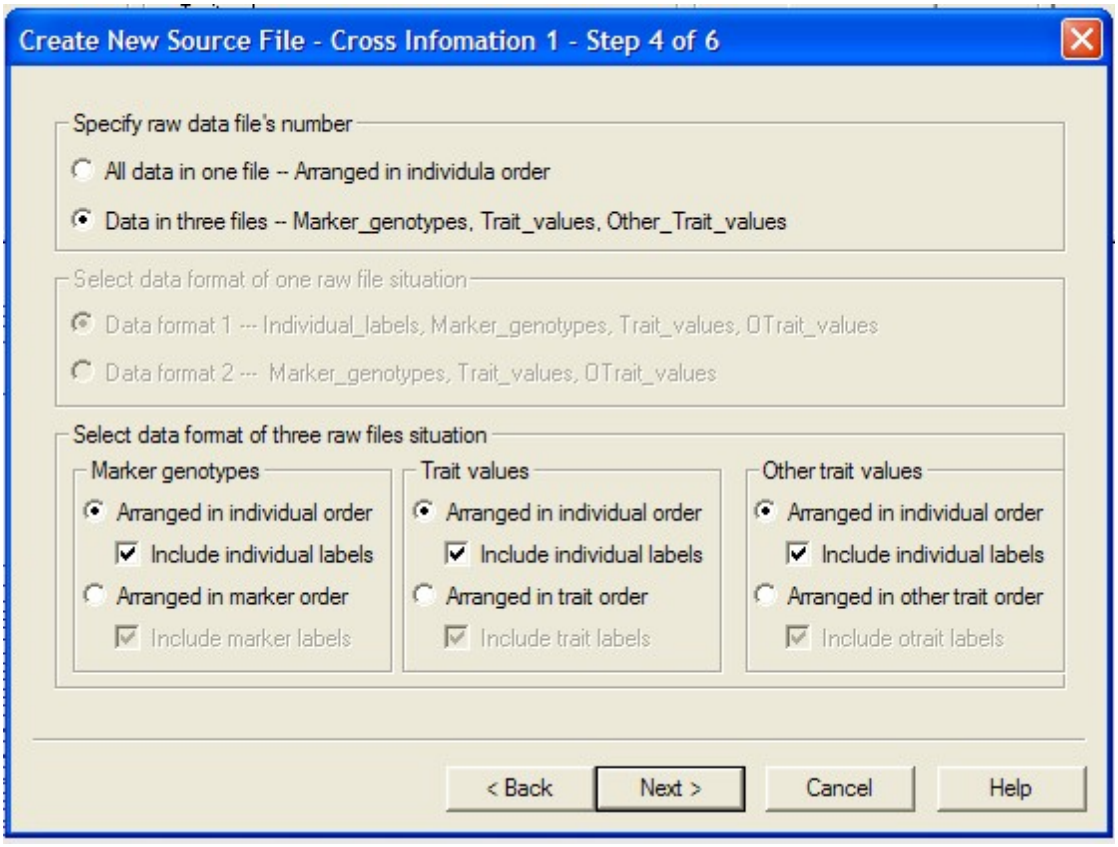
Notepad. Open a blank Notepad file, for use as a holding area for data, provide an editing scratchpad, and so on.

3. Click Next button to accept the values. The Create New Source File – Step 3 of 6 appears.



Click radio button **Labels** to input and send marker label information and click radio button **Positions** to input and send marker positions information. Other operation is similar to above step 2.

4. Click Next button to accept the values. The Create New Source File – Step 4 of 6 appears.



First to specify raw data file's number by click one of the radio button.

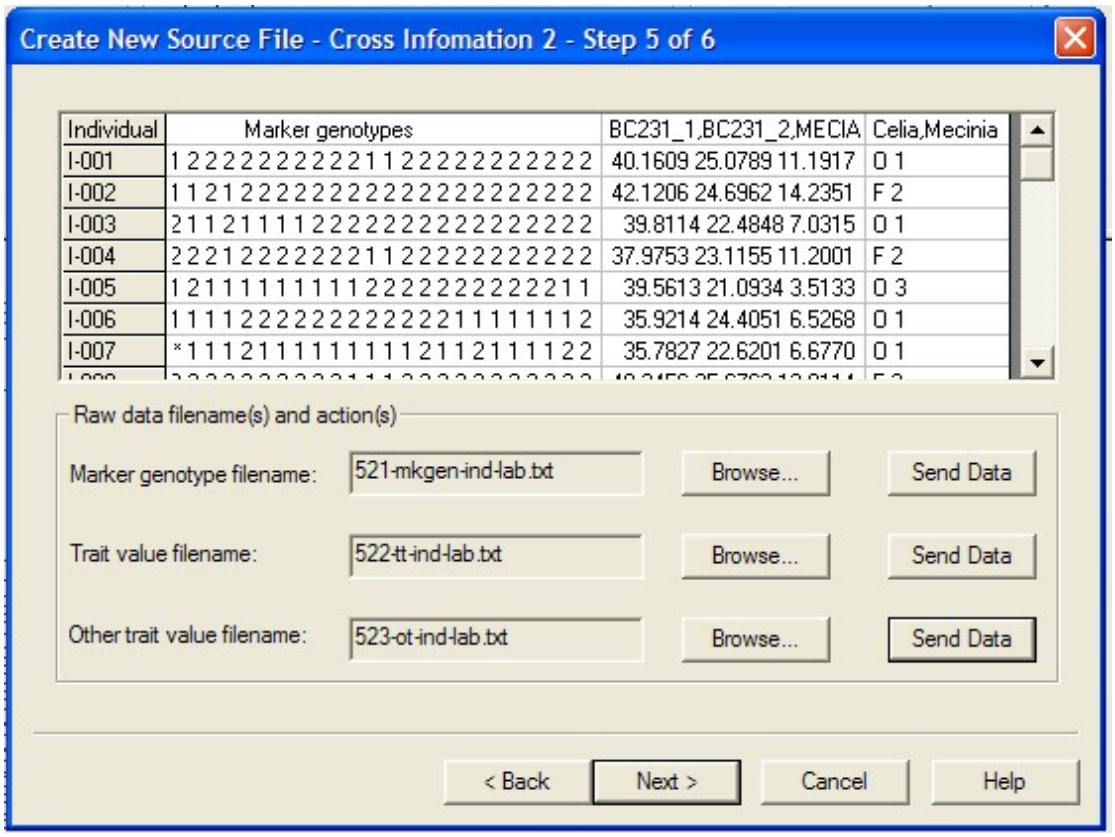
1. All data in one file

Use one file that includes marker genotype, trait, and other trait information. Select **Data format 1** in Select data format of one raw file situation group box if the data include individual label. Otherwise select **Data format 2**. All data must arrange according to individual order.

2. Data in three files

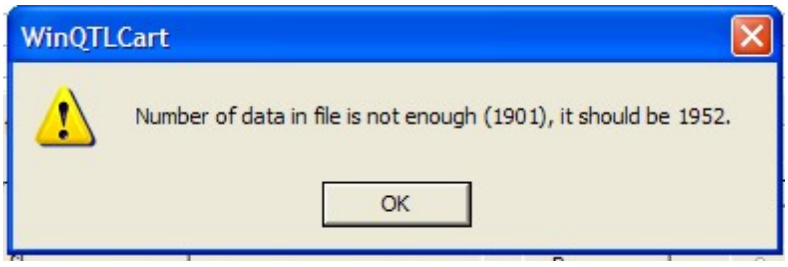
Use three files for marker genotype data, trait data, and other trait data. For each file, you can choose arrange according to individual or according to marker (trait, or other trait) in Select data format of three raw files situation group box. To check corresponding box if there are labels in file.

5. Click Next button to accept the values. The Create New Source File – Step 5 of 6 appears.

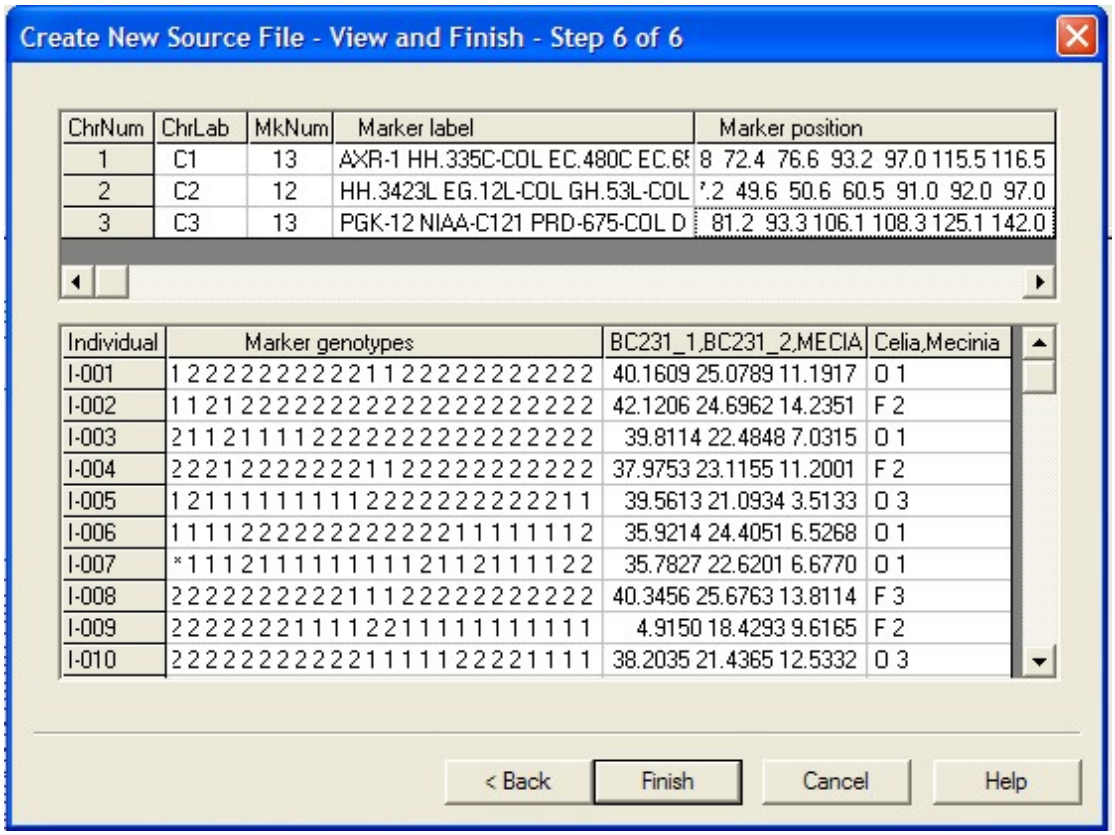


Please notice that the buttons in this dialog is depend on the selections of previous dialog.
For marker genotype information, use **Browse** button to indicate the filename and **Send Data** button to update the Data-Window. It is same for the trait value also. The Browse and Send Data button will be disabled if there is no other trait, Otherwise, you can do the same as trait value.
Only one set of **Browse** and **Send data** button will be enabled if you choose one data file format in the previous dialog.

Note: The Data file's format should be comparable with the setting of previous dialog. For example, if there is no individual label in the genotype data file and you check the **include individual labels** button in previous dialog. Then a warning message box will pop-out when you click the **Send Data** button in **Marker genotype filename** group.



6. Click Next button to accept the values. The Create New Source File – Step 6 of 6 appears.



This is the data summary dialog. The up window is map information and lower window is cross information. Click **Back** button to do the modification if the information is not right. After you check all the necessary data, click **Finish** button WinQTLCart will:

- Verify the data and display error messages if the data file is incomplete or formatted incorrectly.
- Create the .MCD file using the filename stem you entered at the first Create New Source File dialog.
- Show a create .MCD file successful dialog, click OK button to the dialog and display the Main window, with the MCD file you just created loaded into the Source Data form.

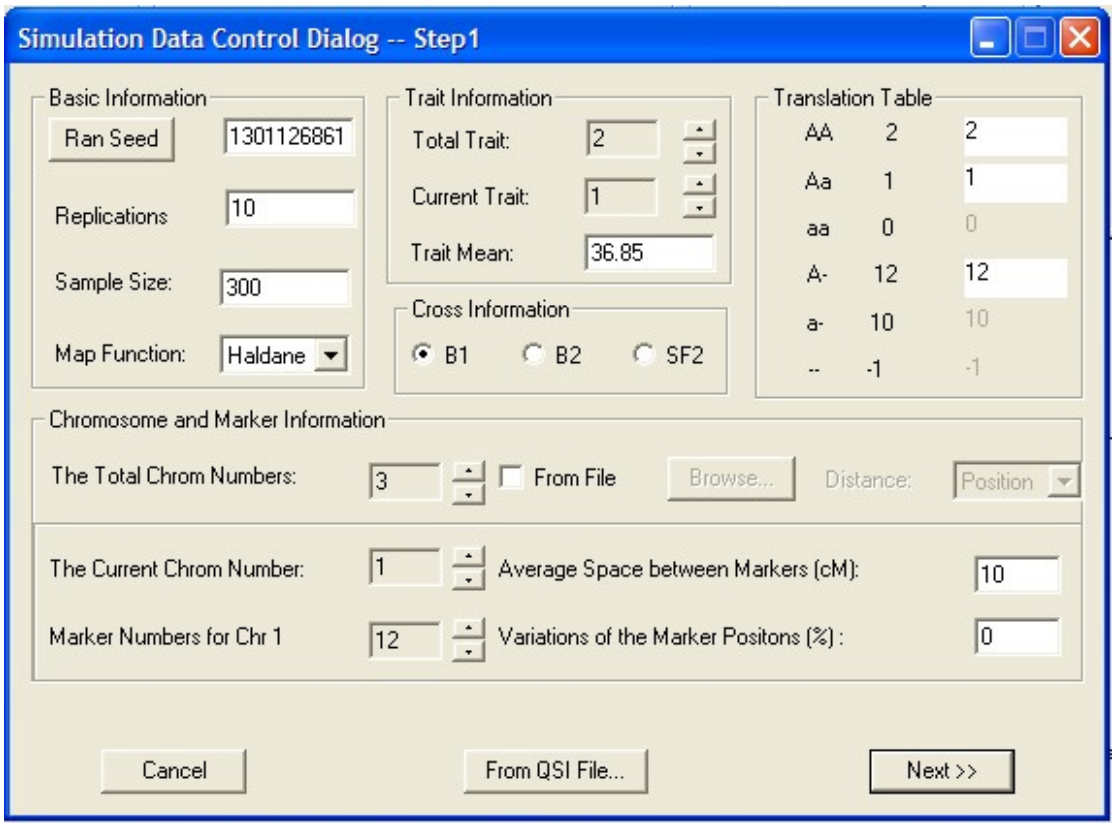
Where to go from here

Select an analysis method 2

Creating simulation data

To use WinQTLCart's results graphing abilities, you need data for it to work on. If you haven't collected any data, though, you can still simulate a dataset that WinQTLCart can work with.

To create a data simulation file, select File>Simulation. This displays the Create Simulation Data series of dialogs.



Fill in the blanks for yourself, or accept the defaults.

Random seed. Click the button to generate a new random seed.

Replications. Produce a file with many replications of simulation data with the same parameter setting. The filename look like ***SimuRep-1.dat and the ***.mcd is the simulation .MCD file.

Sample size. Individual number.

Map function. Haldane (assumes no crossover interference) or Kosambi (assumes some crossover interference).

Total Trait. Indicate how many traits in the simulation data.

Current Trait. You can change the current trait and set parameter of **Trait mean**.

Cross Information. Select an experimental design option. You can select only one option to modify at a time. (At this time, only the three options below are available for selection.)

- B1. Backcross, with 1=parental line to which the F1 line was crossed.
- B2. Backcross, with 2=parental line to which the F1 line was crossed.
- SF2. Selfed intercross line, generation 2.

Translation table. Edit or add tokens you want to use for these genotype markers; you can enter any alphanumeric character(s) as a token.

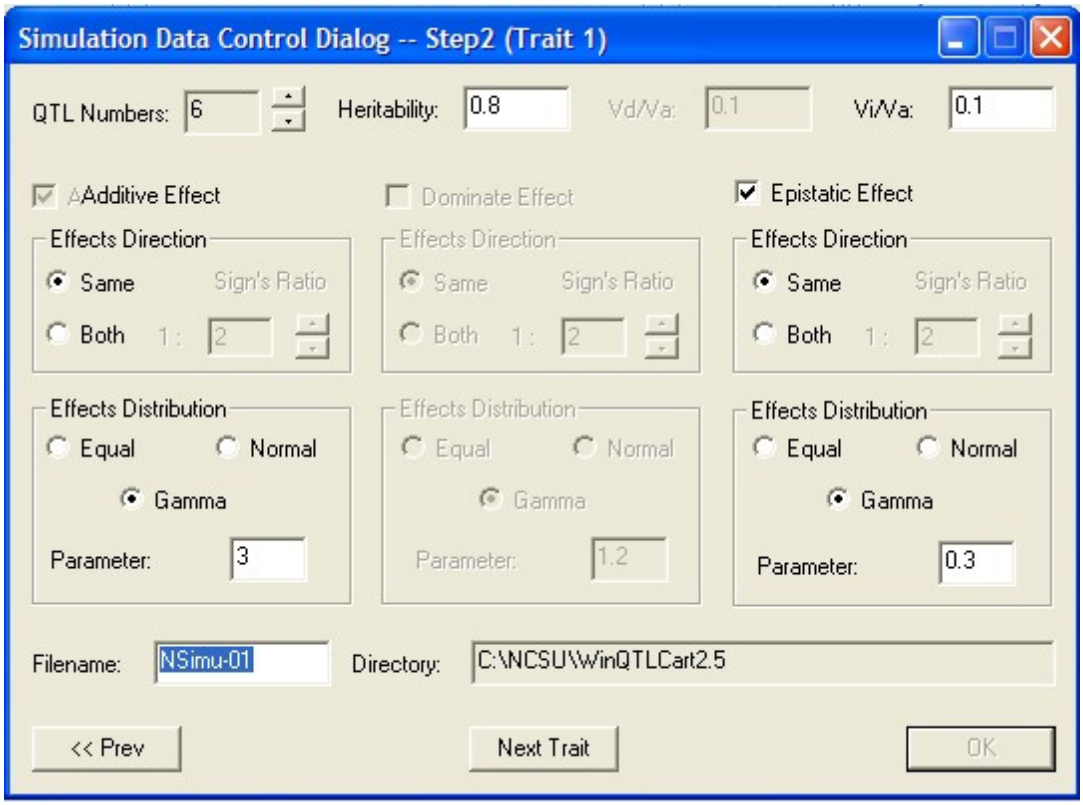
Note *WinQTLCart assumes that the A allele is diagnostic for the High (parental 1) line and the a allele is diagnostic for the Low (parental 2) line. A minus sign (-) means the allele is unknown.*

WinQTLCart uses the numbers (2, 1, 0, 12, 10, -1) to determine how to encode the output of the genotypes. The alphanumeric tokens you enter here indicate how you have coded the markers in the source data. See the topic MCD file format 391 for more information.

Chromosome and marker information

- **Total chromosome number.** The total number of chromosomes. Click the spin dial to the right of this field to select more or fewer.
- **Current chromosome.** Shows the active chromosome you're working on. Click the spin dial to the right of this field to select a new chromosome. You'll see the new chromosome's information in the Distance and Label boxes to the right.
- **Markers for chromosome.** Total number of markers for this chromosome.
- **Average space between markers (cM).** Produces marker positions randomly.
- **Variations of the marker positions (%).** How many deviations are allowed, on average
- Check **Read marker positions from a text file** and click Browse to select a text file containing that information. Also, select a marker distance type.
- **Position.** Indicates that the numbers indicate positions from the left telomere of the current chromosome. This means the numbers should be in ***increasing*** order.
- **Interval.** Indicates that the numbers are for the interval distance after a marker. This means that the ***last number, and the last number only,*** should be ***zero***.

From QSI file. To produce the simulation data by loading a QSI file alone. In Step 3, you can save all simulation parameters into a QSI file.



Filename. The file name of the MCD file that will be created.
Directory. The directory that the simulated MCD file will be created.

Next Trait. Click this button to reach each trait and you can set following parameters.

QTL numbers. Set the number of QTLs to appear in the file.
Heritability. Requires a value in the range 0.0 to 1.0. Default is 0.8.
Vd/Va. Dominant variance/Additive variance
Vi/Va. Epistatic variance/Additive variance

For the **Additive**, **Dominant**, and **Epistatic** effects you want to see in the simulation, you can select the following:

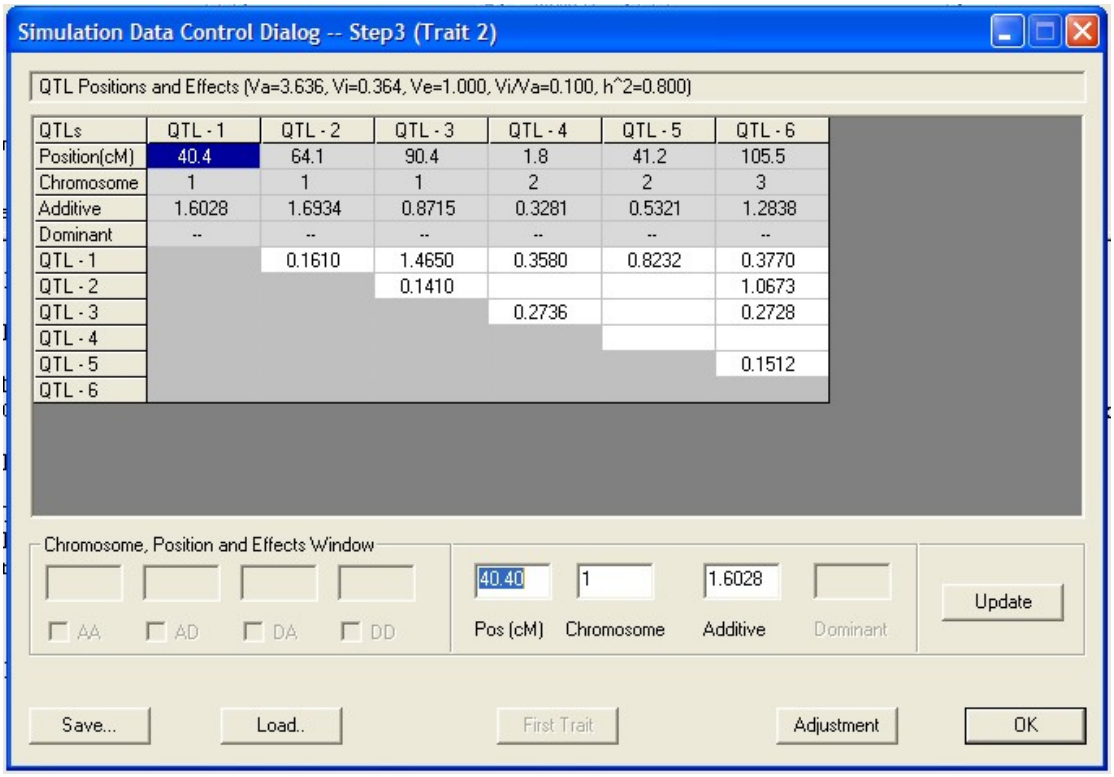
Effects direction

- Same—All values are positive
- Both—Values are positive and negative
- Sign's Ratio—Ratio of negative:positive.

Effects distribution

- Equal—All effects have the same value
- Normal—Values derive from normal distribution
- Gamma—Values derive from gamma distribution
  - Parameter—Value for gamma distribution

Note *For more information on effects distribution and the additive, dominant, and epistatic effects, please refer to the QTL Cartographer's manual.*



After inputting the parameters in the previous two dialogs, the final dialog presents the data simulation model you have built. Click in any cell to see that cell's values represented in the fields below.

Next Trait (or First Trait). Click this button to reach each trait.

Use the fields to edit a cell's contents. Click the **Update** button to write the change to the model.

- Click **Save** to save your model to a .QSI (QTL Simulation Information) file. The current working

directory is selected as the default. (Click the X in the dialog's upper right corner to close without saving.)
- Click **Load t**o load a .QSI file to this window. The file's values replace the values you see in the current window.
- Click **Adjustment** to adjust values for the entire genome.

Click OK to produce the simulation data file. You can then use that file for analysis.

Related topics
Showing QTL information 24

Single-marker analysis

When to use
For quick scanning of the entire genome (all chromosomes) to find best possible QTLs and identify missing (or incorrectly formatted) data. Use single-marker analysis first to ensure your data file is clean; then move on more sophisticated analysis methods, such as Interval Mapping 58 and Composite Interval Mapping 60.

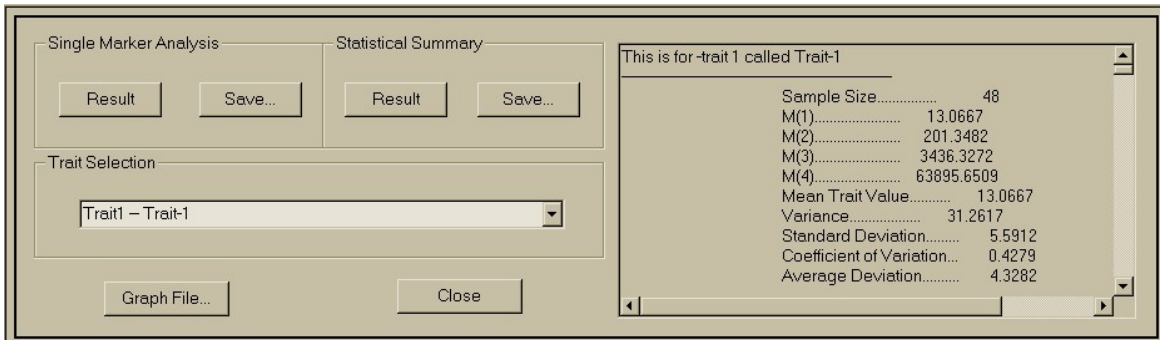
How it works
Single-marker analysis is based on the idea that if there is an association between a marker genotype and trait value, it is likely that a QTL is close to that marker locus.

Comments
Single-marker analysis can be somewhat useful for a quick look at data, but it has been superceded by Interval Mapping and Composite Interval Mapping. IM and CIM are more thorough and accurate indicators of QTL. The prime value of WinQTLCart's single-marker analysis is its identification of missing data that could affect later analysis.

Running a single-marker analysis

1. Open a mapping source data file (an .MCD file) into the WinQTLCart main window.

2. Select Method>Single-Marker Analysis. WinQTLCart analyzes the data and displays the single-marker analysis controls in the form pane. The information pane on the right includes the analysis results.



3. Select a trait for display from the Trait Selection pull-down list. All the traits present in the file will be on the list.

4. For each trait, the information pane on the right displays WinQTLCart's statistical summary of the

file. (You can view this summary in a larger window by clicking the Result button in the Statistical Summary group box, just to the left of the information pane.)

5. In the Single Marker Analysis group box, click Result to view the analysis result for the selected trait. You can change the font used by the display window to make the results easier to read. Click the Save… button in this group to save the marker analysis results to a text file.

6. In the Statistical Summary group box, click Result to view the summary in a larger display window. Click the Save… button to save the statistical results to a text file.

   The statistical summary includes:
   - Basic summary of the data
   - A histogram for the quantitative trait
   - WinQTLCart's summary of missing individuals that should be present, as indicated by the data. If markers show 0% data, there was likely an import problem.
   - Summary of marker segregation
   - Combines LR map QTL and Q stats

7. Click the Graphic File… button to save the results to a QTL mapping result file (*.QRT). You can open this .QRT file later to view the results as a graph.

8. Click Close to end the single-marker analysis session and return to the Form View of Source Data.

Qstats show some important results:
- Tells you that the data were imported correctly. If a marker has 0% data, that indicates a problem (likely an import problem). For example, the original marker may be *Marker_1, but QTL sees that as *Marker-1, and the values won't match.
- Tests for segregation distortion

Setting threshold levels (IM & CIM)

Both Interval Mapping 58 and Composite Interval Mapping 60 use threshold values to determine QTLs. If you don't know the threshold value for a trait, you can have WinQTLCart find them out and use them as inputs for the QTL search.

The threshold levels control the rate of Type 1 errors (false positives). A lower threshold value means more false positives; but a higher value means you may miss more QTLs.

When you look at a result graph 17, the horizontal line you see running across the graph is the threshold level. When the graph peaks over the threshold level, that is good evidence for a QTL. So, setting the threshold level in this form controls how high or low that line will sit on the graph. Too high and you'll see no QTLs; too low and you'll see too many. (The threshold value can be changed later in the Graph window.)

Getting a very accurate threshold level may take a long time. Recommendations are included in the threshold procedures.

1. Follow the instructions for the form you're working with (either IM or CIM). Be sure to select a trait or all traits for which you want to set a threshold level.

2. The Threshold group box on the right of the form controls inputting or obtaining mapping threshold

values for each trait. Select a different trait number to see the threshold value of that trait.

3. Select whether you want to set the threshold levels manually or have WinQTLCart determine the threshold via permutations.

If you...	Do this...	Comments
Know the threshold values you want to use	Input them into the Manual Input 57 box	
Don't know the threshold values you want to use	Select By Permutations 57 to have WinQTLCart calculate them.	Permutations take a long time to run. If 1 permutation runs for 10 seconds, than 1000 permutations will take 2-3 hours.

Setting threshold levels manually

This analysis occurs before you start the QTL analysis.

Use this procedure to set the threshold for each trait manually. This is useful if you know the value for each trait. Alternatively, select All Traits and apply this threshold setting to all the traits.

1. From the Trait Selection pull-down, select a trait to find the threshold value for that trait. (The All Traits selection is available for permutations only.)
2. Click the By Manual Input option.
3. Input a threshold value into the box.
4. Optional: Click Set as Default if you want to assign the threshold value you entered to all traits.

Setting threshold levels via permutations

This analysis occurs before you start the QTL analysis.

Setting values via permutations allows WinQTLCart to empirically estimate the genome-wide significance threshold. Use this procedure if you don't know the value and you want WinQTLCart to find the optimal threshold.

Threshold values will be filled in automatically for every trait according to significance level.

Following threshold analysis, WinQTLCart will display the results in the Data pane and save them in a text file. The text file's name is displayed in the form after the analysis completes.

1. From the Trait Selection pull-down, do one of the following:
   - Select one trait to find the threshold value for that trait.
   - Select All Traits to find threshold values for all traits.
2. Click the By Permutations option.
3. Enter a value for Permutation Times to set the permutation repeating times.

   Note *If you want a quick run, use the default value of 300. But if you're going to publish, use the 1000 value; this will take several hours to run but will yield very precise results. We recommend starting this analysis before you leave for the day and letting it run overnight.*

4. Enter a value for Significance Level.
5. Click OK to begin threshold analysis.

Note *You cannot update the All Traits checkbox. WinQTLCart checks or clears this box based on whether you select one or all traits from the drop down list.*

After you click OK, WinQTLCart displays a countdown showing its progress through the file. When the threshold analysis is complete, WinQTLCart will display the results in the Data pane and display the results file's name. (WinQTLCart saves the file to the current working directory 25.)

After setting the threshold value, move on to finish the IM 58 or CIM 60 analysis procedure.

Interval Mapping

What it is
Interval mapping (IM) is an extension of single-marker analysis 55. In single-marker analysis, only one marker is used in QTL mapping but effects are underestimated and the QTL position cannot be determined. Interval mapping provides a systematic way to scan the whole genome for evidence of QTL.

IM uses two observable flanking markers to construct an interval within which to search for QTL. A map function (either Haldane or Kosambi) is used to translate from recombination frequency to distance or vice visa. Then, a LOD score is calculated at each increment (walking step) in the interval. Finally, the LOD score profile is calculated for the whole genome. When a peak has exceeded the threshold value, we declare that a QTL have been found at that location.

When to use it
IM is a good general standard to use for all datasets.

Use it in combination with or as part of a process including
You may wish to start with a single-marker analysis 55 and then run IM to further refine the analysis.

High-level process
Here's a quick overview of how to use WinQTLCart's IM implementation. The first few times you run this analysis, go with the WinQTLCart default values for the form's parameters. The defaults provide the best all-around parameter settings, especially for initial analysis sessions.

1. Select the IM analysis method.
2. Select the chromosome(s) and trait(s) you want to analyze.
3. Select a threshold level to apply to the selected trait(s). Select either By manual input 57 (the WinQTLCart default) or By permutations 57 (to have WinQTLCart determine an optimum threshold). See Setting the threshold level 56 for more information on the impact of each of these choices.
4. Click OK to start the calculations for the threshold level.
5. Following threshold calculation, set IM form parameters 58. Select a walk speed in cM. It's recommended you use the same walk speed for your entire dataset. Don't reset the walk speed between runs or your results will not be comparable.
6. Click Start to begin the analysis.

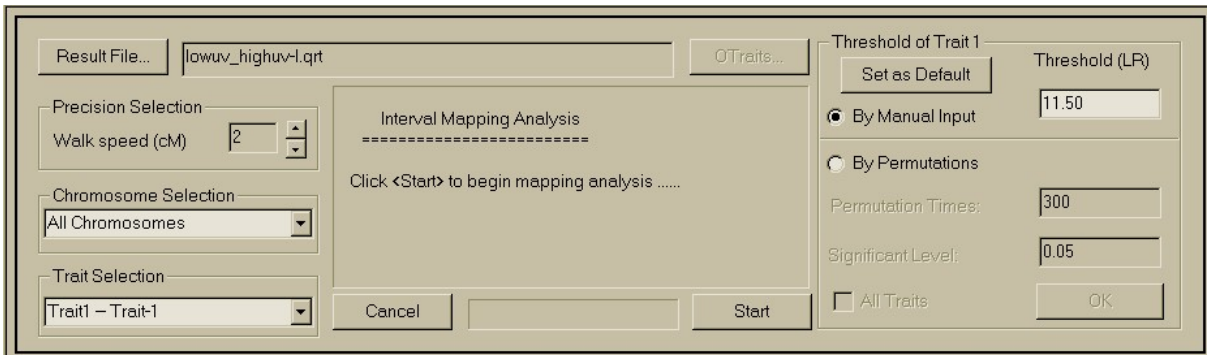
Running interval mapping analysis

WinQTLCart provides default values for the parameters in this form. The defaults provide the best all-around parameter settings, especially for initial analysis sessions.

Interval mapping analysis uses WinQTLCart mapping source data files (.MCD files). Use WinQTLCart's import commands to move your source data files from text to .MCD format.

1. Open a source data file into the WinQTLCart main window.

2. Select Method>Interval Mapping. WinQTLCart displays the interval mapping analysis controls in the form pane.



3. Click Result File… to select the location of and to name the .QRT file that will be created when the analysis is complete.

4. Click the OTraits… button to enter other trait numbers or number ranges. WinQTLCart will use these as a co-factor in the analysis. (WinQTLCart's default is no OTraits.)

   Note *OTraits is another term for "categorical traits." Use OTraits for background control as nuisance factors we want to account for.*

5. The Walk speed (cM) is the genome scan interval. and the default is 2. Click the spin dial beside the Walk speed value to increase or decrease the walk speed by .5 increments.
   - Increasing the walk speed (greater than 2) means less precision but the analysis takes less time.
   - Decreasing the walk speed (less than 2) yields a more precise result but will take more time.

   You should set the walk speed value once for the entire dataset. A single walk speed establishes a consistent norm against which the data can be graphed. If you change the walk speed between mapping runs, the graph displays will be skewed. (If you want to check your data against a different walk speed, create a separate directory for your data files, and then run the new walk speed against those files.)

6. Select one or all chromosomes to include in the analysis.

7. Select one or all traits to include in the analysis. The Traits you select may change the value of the Threshold controls on the right side of the form.

8. Set the threshold level via either manual input or permutations. For more information, see Setting threshold levels (IM & CIM) 56.

9. Click Start to begin QTL mapping analysis.

Following the analysis, WinQTLCart will:

- Create a QTL mapping result file (*.QRT) and open it in the Graph window
- Create a QTL summary information file using the EQTL function.
- Display a confirmation box asking if you want to display QTL summary information in the Main window's Data pane.

Composite Interval Mapping

What it is
Composite interval mapping (CIM) adds background loci to simple interval mapping 58 (IM). CIM fits parameters for a target QTL in one interval while simultaneously fitting partial regression coefficients for "background markers" to account for variance caused by non-target QTL.

"In theory, CIM gives more power and precision than simple IM because the effects of other QTL are not present as residual variance. Furthermore, CIM can remove the bias that would normally be caused by QTL that are linked to the position being tested."

Background markers are usually 20-40cM apart.

High-level workflow
Here's a quick overview of how to use WinQTLCart's CIM implementation. The first few times you run this analysis, go with the WinQTLCart default values for the form's parameters. The defaults provide the best all-around parameter settings, especially for initial analysis sessions.

1. Select the CIM analysis method.
2. Select the chromosome(s) and trait(s) you want to analyze.
3. Select a threshold level to apply to the selected trait(s). Select either By manual input 57 (the WinQTLCart default) or By permutations 57 (to have WinQTLCart determine an optimum threshold). See the Setting the threshold level 56 topic for more information on the impact of each of these choices.
4. Click OK to start the calculations for the threshold level. This may take from several minutes to several hours to run.
5. Following threshold calculation, set CIM form parameters 60. Select a walk speed in cM. It's recommended you use the same walk speed for your entire dataset. Don't reset the walk speed between runs or your results will not be comparable.
6. Click Start to begin the analysis. The analysis may take from 20 minutes to several hours to run.

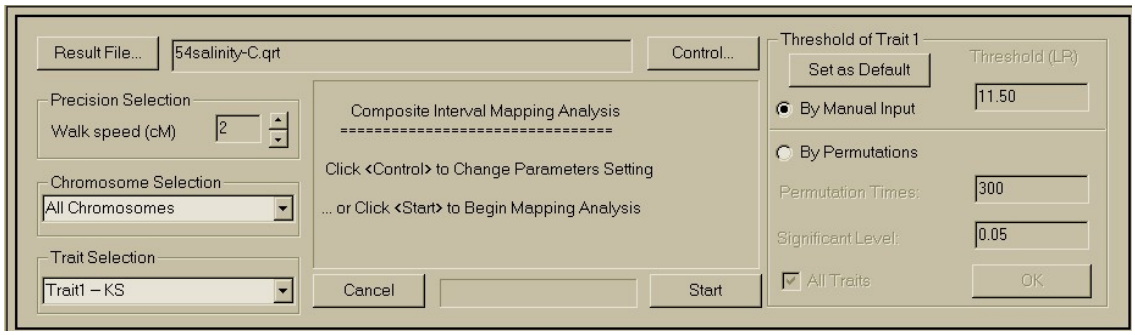
Running composite interval mapping analysis

WinQTLCart provides default values for the parameters in this form. The defaults provide the best all-around parameter settings, especially for initial analysis sessions.

Composite interval mapping analysis uses WinQTLCart source data mapping files (.MCD files). Use WinQTLCart's import commands to move your source data files from text to .MCD format.

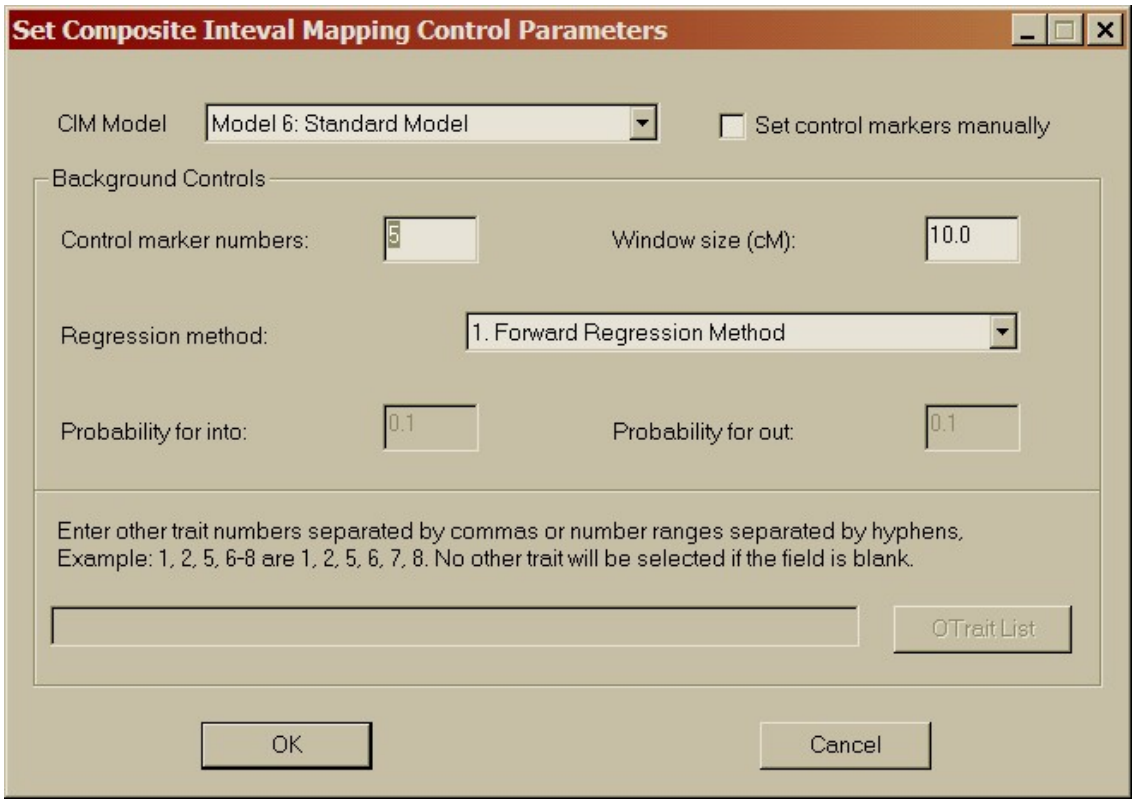
1. Open a source data file into the WinQTLCart main window.

2. Select Method>Composite Interval Mapping. WinQTLCart displays the CIM analysis controls in the form pane.



3. Click Result File… to select the .QRT file you want to create.

4. Click the Control… button to display the Set CIM Control Parameters dialog.



4a. For the CIM Model field, specify the markers to be used as cofactors in the CIM analysis:
- **Model 1: All Marker Control**—Use all the markers to control for the genetic background.
- **Model 2: Unlinked Marker Control**—Use all unlinked markers to control for the genetic background.
- **Model 6: Standard Model**—The default model that selects certain markers as control markers by using additional parameters: control marker number and window size. Selecting this model requires you to fill in extra fields on the dialog: Control marker numbers, Window size (cM), and Regression method selection (all explained below).

4b. Click **Set control markers manually** if you do not want WinQTLCart to automatically select the control markers. This will display a dialog box ***after*** you start the analysis so that you can

manually select the control markers. Skip to step 10 for a description of this dialog box.

The **Background Controls** group box specifies the number of background controls and regression type WinQTLCart should use in applying the selected CIM model.

4c. **Control marker numbers**—Enter the number of markers to control for the genetic background. WinQTLCart will use up to the number of markers entered here.

4d. **Window size (cM)**—Enter the window size in centiMorgans. The window size will block out a region of the genome on either side of the markers flanking the test site. Since these flanking regions are tightly linked to the testing site, if we were to use them as background markers we would then be eliminating the signal from the test site itself.

If the control marker number is…	And if the window size is…	This is the result
The total number of markers	0.0	Model 6 reduces to Model 1
The total number of markers	Large (such as the size of the largest chromosome)	Model 2
Zero	N/a	Model 3

Recommendations

- Model 6 is good for starting an analysis. Start with the default values of 5 for control markers and 10 for window size.
- Increasing the number of control markers will allow better resolution for mapping linked QTLs.

4e. **Regression method selection**—Select a method.

- 1: Forward Regression
- 2: Backward Regression
- 3: Forward & Backward

4f. **Probability for into:, Probability for out:**

4g. If the OTrait number field is enabled, enter other trait numbers and their ranges to be included in the model.

Note *OTraits is another term for "categorical traits." Use QTraits for background control as nuisance factors we want to account for.*

4h. Click OK to close the dialog and return to the CIM analysis form.

5. The Walk speed (cM) default is 2. The walk speed is the genome scan interval. Click the spin dial beside the Walk speed value to increase or decrease the walk speed by .5 increments.
   - Increasing the walk speed (greater than 2) means less precision but the analysis takes less time.
   - Decreasing the walk speed (less than 2) yields a more precise result but will take more time.

   You should set the walk speed value once for the entire dataset. A single walk speed establishes a consistent norm against which the data can be graphed. If you change the walk speed between runs, the graph displays will be skewed. (If you want to check your data against a different walk speed, create a separate directory for your data files, and then run the new walk speed against those files.)

6. Select one or all chromosomes to include in the analysis.

7. Select one or all traits to include in the analysis. The Traits you select may change the value of the Threshold controls on the right side of the form.

8. Set the threshold level via either manual input or permutations. For more information, see Setting threshold levels (IM & CIM) 56.

9. Click Start to begin QTL mapping analysis. WinQTLCart will open a Save As dialog for you to save the result file that will be created.

10. If you selected **Set control markers manually** in step 4b, then WinQTLCart will display the Select CIM Control Markers dialog box. Enter or edit the marker numbers you want to using the text box; separate each number with a space. Click on the marker row's cells to toggle their display in the text box.

When the analysis is complete
WinQTLCart will

- Create a QTL mapping result file (*.QRT) and open it in the Graph window
- Create a QTL summary information file using the EQTL function.
- Display a confirmation box asking if you want to display QTL summary information in the Main window's Data pane.

Multiple Interval Mapping

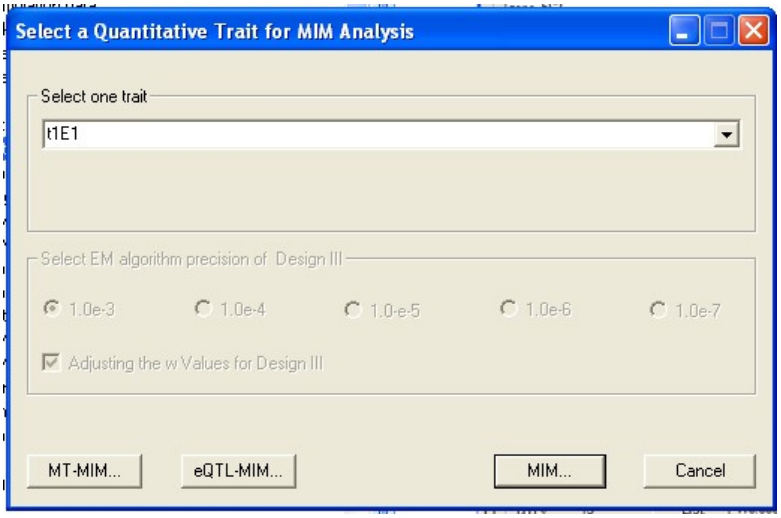
What it is
Multiple interval mapping (MIM) uses multiple marker intervals simultaneously to fit multiple putative QTL directly in the model for mapping QTL. The MIM model is based on Cockerham's model for interpreting genetic parameters and the method of maximum likelihood for estimating genetic parameters. MIM is well suited to the identification and estimation of genetic architecture parameters, including the number, genomic positions, effects and interactions of significant QTL and their contribution to the genetic variance.

High-level process
Here's a quick overview of how to use WinQTLCart's MIM implementation:

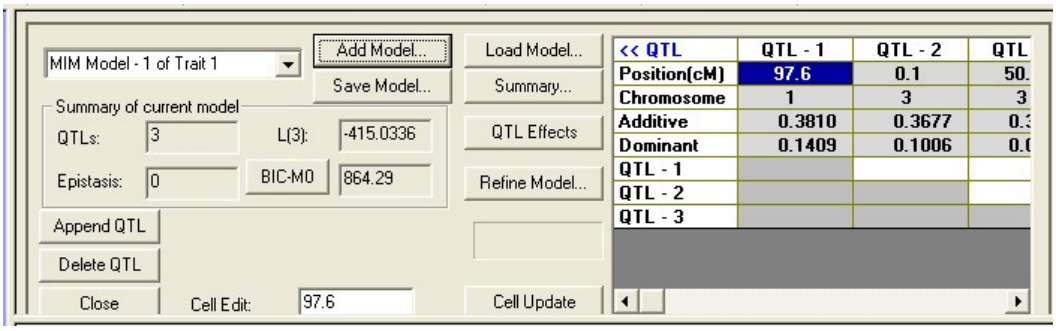
1. Select the MIM analysis method.
2. Pick a trait you want to work with. (MIM works with only one trait at a time.)
3. Decide if you want to create a model using WinQTLCart's default search procedures or an alternative (such as Forward, Backward, or CIM).
4. Run the analysis to generate the model.
5. Refine the model as needed by editing individual cells in the model, adding or deleting QTL, searching and testing QTLs or epistatics, and re-estimating. This part of the analysis can iterate for as long as you want to search for QTLs.
6. Save the model as a .MDS file (or as a result file using the Refine Model function).

About the MIM form

From the Source Data form, open a source data file and select Multiple Interval Mapping as the analysis method. If there is more than one trait in the dataset, the Select Trait for MIM Analysis dialog appears. Select a trait from the drop down list and Click MIM... button to open MIM form.



The MIM form appears. Before you can use this form, you need to load or create a MIM analysis model. You can open existing files containing MIM model parameters or you can use WinQTLCart to create a model. The following screen shot shows the MIM form with a model loaded and analyzed.



Model drop down list. Contains the list of MIM models to be used for the analysis. You can create or load several different models for selection.

New Model / Add Model. Have WinQTLCart create a new inital MIM model or create additional MIM model for analysis.

Save Model. Save the model you've created or modified to an .MDS file.

Load Model. Load an existing MIM model parameters file (.MDS). Click the button to display an Open dialog; navigate to the .MDS file containing the parameters and click OK.

Summary. Click to create a text summary file and a graph result file (.QRT). At the prompts, confirm you want to create the files. WinQTLCart by default saves them to the current working directory 251, but you can specify a different location and filename.

Note *The summary file information includes position, likelihood ratio and effect of each QTL, epistatic effects of QTL, partition of the variance explained by QTL (main and interaction effects), and estimates of genotypic value of individuals based on the model.*

Parameters for current model

QTLs. Number of QTLs in model

Epistasis. Number of epistatic genes in model
L(k). Likelihood of the mode, k is the QTL number.
BIC. Bayesian Information Criteria (BIC) value of the mode.

QTL Effects. Click to test additive, dominant and epistatic effects. The data pane under the form will show the test results. You can select the text and then Edit>Copy to copy it to the clipboard.

Refine Model.... Select an option and click OK to refine the model 70 's parameters.

Add QTL. Adds a QTL to the model.

Del QTL. Select a QTL column and click Del QTL to delete that QTL from the model.

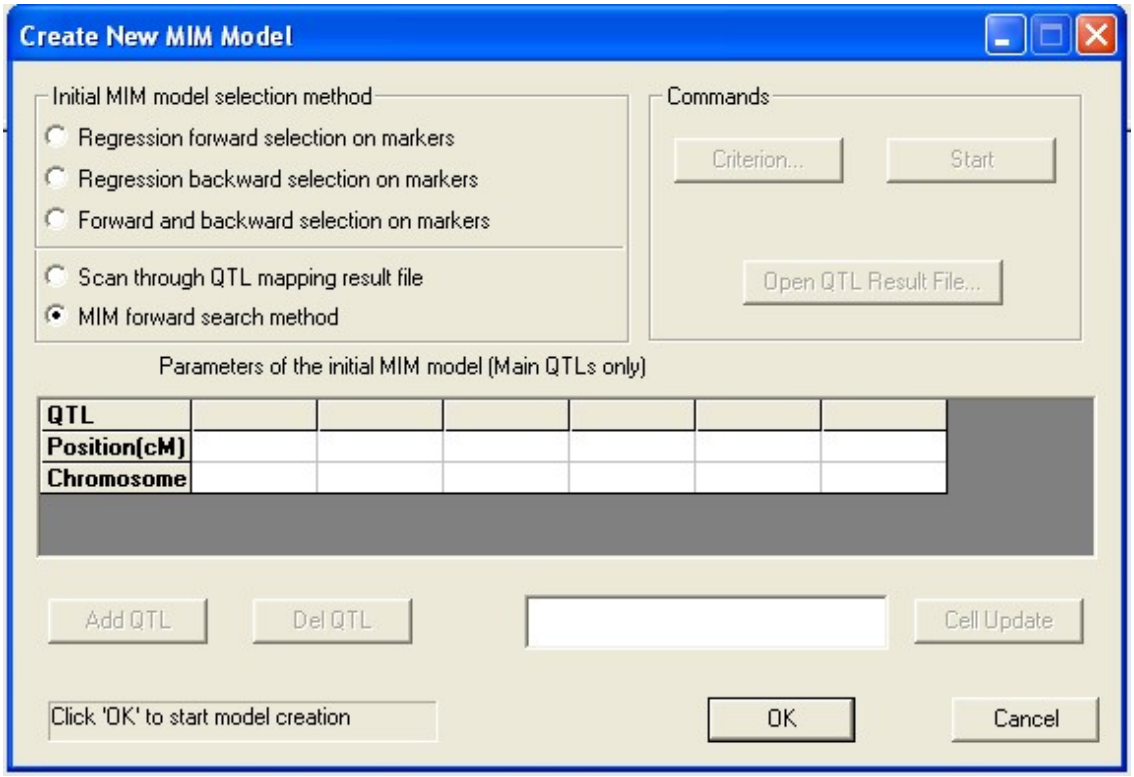
Cell Edit / Cell Update. Click on a cell in the model to select it and then update its value in this field. Click the Cell Update button to write the value to the cell.

Close. Close the MIM form and return to the Source Data form. If you have not saved your work, you can save your work at this time.

The Model. Occupies the right half of the form. Click the blue <<QTL cell to expand the model so it fills the form pane; click the QTL>> cell to re-display the MIM form.

Creating MIM initial model

Click the New Model (or Add Model) button on the MIM form. The following Create New MIM Model dialog appears. At the Create New MIM Model dialog, select an enabled option from the Initial MIM model selection method group box.



Regression options 66

- Regression forward selection on markers. Enables the Criterion… button
- Regression backward selection on markers. Enables the Criterion… button
- Forward and backward selection on markers. Enables the Criterion… button

CIM search option 68

- Scan through composite interval mapping. Enables the Control… and From File… buttons. (From File… displays only after you select this option.)

MIM search option 69

- MIM forward search method. Enables the OK button

After finishing the initial model creation, the MIM form redisplays with the buttons enabled, the Parameters group fields populated, the new model available in the drop down list, and the model values on the right . The Parameters fields are now populated.

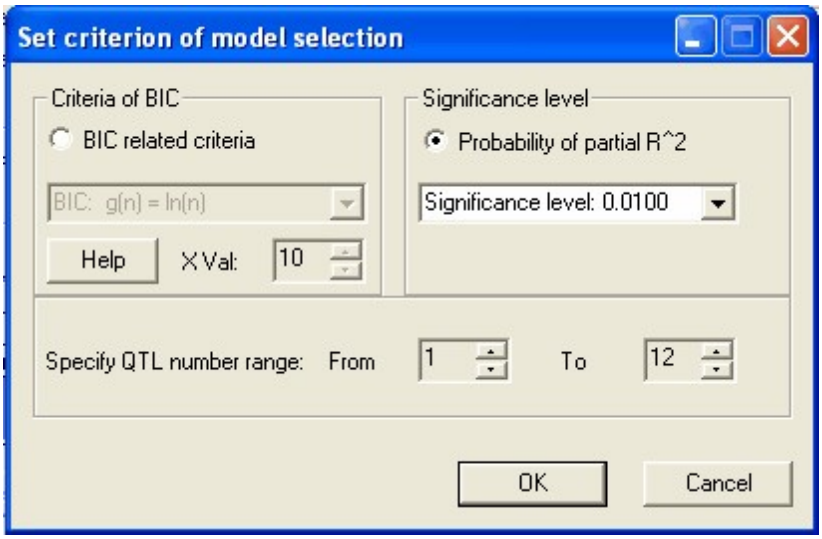
Note *To see the entire model without scrolling, click the blue <<QTL cell. To return to the MIM form, click the blue QTL>> button.*

Where to go from here
From here, you can refine the MIM model 70, manually edit the model by clicking the Add QTL and Del QTL buttons, or click in the model field to change the value of Position, Chromosome, Additive, or epistatic values. Click Save Model… to save the model as a .MDS file.

Related topics
About the MIM form 63
Refining the MIM model 70

Regression options

In the Create New MIM Model dialog: If enabled, click the Criterion… button to display the Set Criterion dialog box.



- Select the **BIC-related criteria** option and select a criterion from the drop down list. Use the spin dial to select an appropriate X Value. The Help button displays a message box explaining the meanings of the BIC-related criteria.
- Select the **Probability of partial R^2** and select a significance level from the drop down list.

1. Specify a QTL number range using the spin dials and click OK. The dialog closes and you're returned to the Create New MIM Model dialog.

2. Click Start to begin the search. You may see the WinQTLCart Main window's status bar underneath the dialog begin filling in as the search progresses. When the search is complete, the model is presented in the cells.

3. To manually edit the model:
   - Click Add QTL or Del QTL
   - Click on a cell and enter a new value in the Cell editing box. Click the Cell Update button to enter the new value.

4. Click OK to accept the parameters, close the dialog and return to the MIM form. The model you created is now displayed in the form.

Where to go from here
From here, you can refine the MIM model 70, manually edit the model by clicking the Add QTL and Del QTL buttons, or click in the model field to change the value of Position, Chromosome, Additive, or epistatic values. Click Save Model… to save the model as a .MDS file.

Related topics
About the MIM form 63
Refining the MIM model 70

CIM search option

In the Create New MIM Model dialog: If enabled, click the Criterion… button to display the Set Composite Interval Mapping Control Parameters dialog box.

1. For the CIM Model field, specify the markers to be used as cofactors in the CIM analysis:
   Model 1: All Marker Control. Use all the markers to control for the genetic background.
   Model 2: Unlinked Marker Control. Use all unlinked markers to control for the genetic background.
   Model 6: Standard Model. The default model that selects certain markers as control markers by using additional parameters: control marker number and window size. Selecting this model requires you to fill in extra fields on the dialog: Control marker numbers, Window size (cM), and Regression method selection (all explained below).

2. Click **Set control markers manually** if you do not want WinQTLCart to calculate the number of control markers. This will display a dialog box after you start the analysis so that you can manually select the control markers. Skip to the end of this topic for a description of this dialog box.

3. **Control marker numbers**. Enter the number of markers to control for the genetic background. WinQTLCart will use up to the number of markers entered here.

4. **Window size (cM).** Enter the window size in centiMorgans. The window size will block out a region of the genome on either side of the markers flanking the test site. Since these flanking regions are tightly linked to the testing site, if we were to use them as background markers we would then be eliminating the signal from the test site itself.

If the control marker number is…	And if the window size is…	This is the result
The total number of markers	0.0	Model 6 reduces to Model 1
The total number of markers	Large (such as the size of the largest chromosome)	Model 2
Zero	N/a	Model 3

Recommendations

- Model 6 is good for starting an analysis.
- The default values of 5 for control markers and 10 for window size should be good starting points for Model 6.
- Increasing the number of control markers will allow better resolution for mapping linked QTLs.

5. **Regression method selection.** Select a method:
   1: Forward Regression
   2: Backward Regression
   3: Forward & Backward

6. **Probability for into:, Probability for out:.**

7. If the trait number field is enabled, enter trait numbers and their ranges to be included in the model.

8. Click OK to close the dialog and return to the Create New MIM dialog.

9. Click the **Criterion** button. The Set Threshold dialog appears. Enter a threshold value or click Default to accept the default value (recommended). Click OK to accept the value and close the dialog and return to the Create New MIM dialog.

10. Click the Start button to begin the search. The CIM form may display during the calculations. When the search is complete, the model is presented in the cells.

11. To manually edit the model:
    - Click Add QTL or Del QTL
    - Click on a cell and enter a new value in the Cell editing box. Click the Cell Update button to enter the new value.

12. Click OK to accept the parameters, close the dialog and return to the MIM form.

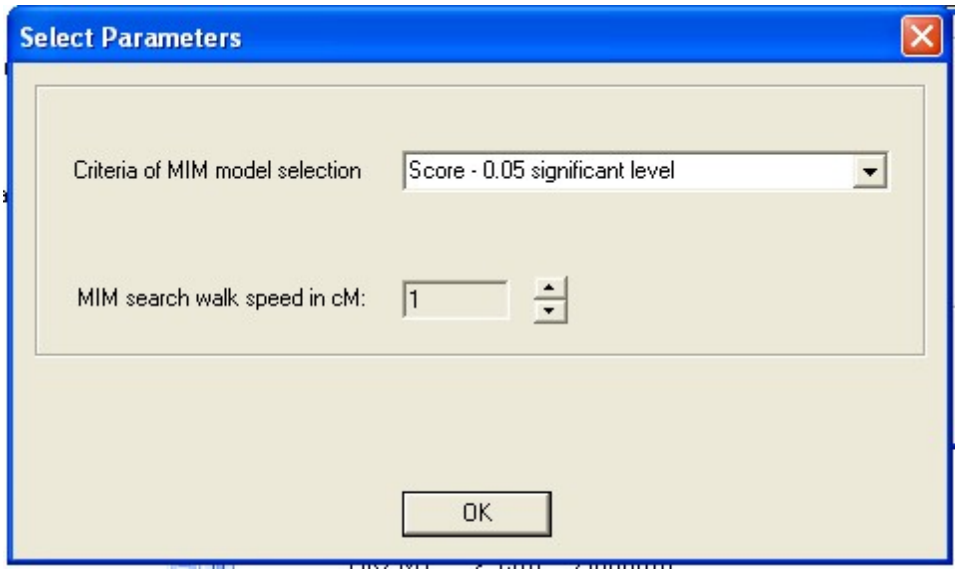
If you selected **Set control markers manually** in step 2, then WinQTLCart will display the Select CIM Control Markers dialog box. Enter or edit the marker numbers you want to using the text box; separate each number with a space. Click on the marker row's cells to toggle their display in the text box.

Where to go from here
From here, you can refine the MIM model 70, manually edit the model by clicking the Add QTL and Del QTL buttons, or click in the model field to change the value of Position, Chromosome, Additive, or epistatic values. Click Save Model... to save the model as a .MDS file.

Related topics
About the MIM form 63
Refining the MIM model 70

MIM search option

1. At the Select Parameters dialog, select a model selection criteria from the drop down list:



BIC-M0 ---> c(n) = ln(n)

AIC ---> c(n) = 2
BIC-M1 ---> c(n) = 2ln(ln(n))
BIC-M2 ---> c(n) = 2ln(n)
BIC-M3 ---> c(n) = 3ln(n)
BIC-X ---> c(n) = 10*X*ln(n)
Score - 0.05 significant level
Score - 0.10 significant level
Score - 0.20 significant level
Score - X significant level

Note: The first 6 options are BIC search criteria. BIC = n*ln(Q*Q)+p*c(n)
n: sample size, Q*Q: residula varance of model, p: regressor (marker) number
Choose last 4 options (Score), WinQTLCart will use score statistics (not LR) to do the forward search for both main and epistatic QTLs as initial MIM model.

2. Click the spin dial beside MIM walk speed in cM to select the walk speed. The smaller the number, the more precise the model, but the longer the analysis will take. (We recommend accepting the default value.)

3. Click OK. WinQTLCart builds the model based on the trait and the model parameters you selected. Under the Cancel button in the form, WinQTLCart displays its progress as it works through the file. The process may take several minutes or several hours, depending on your data. You can safely minimize the WinQTLCart window and work on other apps in the foreground.

4. The MIM form redisplays with the buttons enabled, the Parameters group fields populated, the new model available in the drop down list, and the model values on the right . The Parameters fields are now populated.

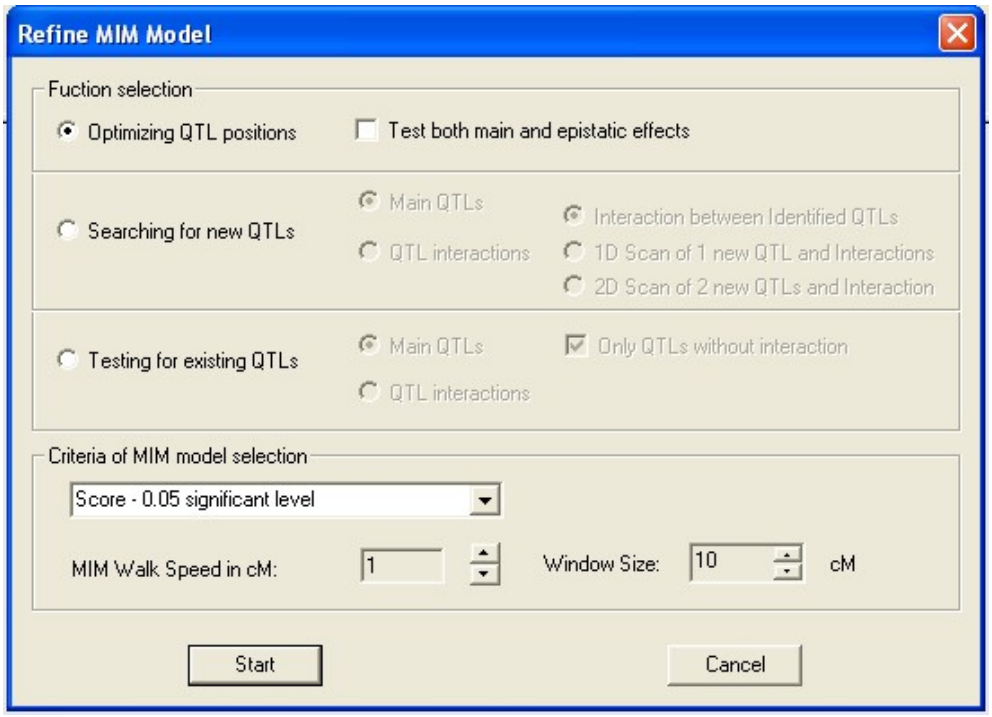
Note *To see the entire model without scrolling, click the blue <<QTL cell. To return to the MIM form, click the blue QTL>> button.*

Where to go from here
From here, you can refine the MIM model 70, manually edit the model by clicking the Add QTL and Del QTL buttons, or click in the model field to change the value of Position, Chromosome, Additive, or epistatic values. Click Save Model… to save the model as a .MDS file.

Related topics
About the MIM form 63
Refining the MIM model 70

Refining the MIM model

After you've specified the model and criteria for the search, the model and its values are loaded into the MIM form. At this point, you can further refine the model. From the MIM form, click **Refine Model** to display the Refine MIM Model dialog.



At the refine MIM model dialog, select a model selection criteria from the drop down list. Choose the first 6 options, WinQTLCart will do search, test, or optimizing in the principle of LR test and use BIC as criteria. By select the last 4 options, WinQTLCart will use score statistics test and certain significant level as search, test and optimizing criteria.

1. Optimizing QTL positions

Move main QTLs one by one along the chromosome to maximize LR or Score statistics (choose the first 6 options is LR and otherwise is score statistics). Check box **Test both main and epistatic effects** is only worked in score statistics testing. By check this check box, both main QTL and its interaction with other QTL(s) are considered in score statistics calculation.

2. Searching for new QTLs

Main QTLs - Search for new main QTL(s) using LR or Score statistics test.
QTL interactions:
Interaction between Identified QTLs - Try to find more interaction among existing main QTLs.
1D Scan of 1 new QTL and Interactions - Search one new main QTL plus interaction between the new QTL and QTL in the model by test the interaction effect only. Aavailable in Score statistics test situation.
2D Scan of 2 new QTL and Interaction - Search two new main QTLs plus interaction between them by test the interaction effect only. Aavailable in Score statistics test situation.

3. Testing existing QTLs

Main QTLs - Test each main QTL to see it is significant or not. The QTL will be deleted from the MIM model if it's not significant.
In Score statistics test, to check **Only QTLs without interaction** check box will do test

only on those main QTL(s) that have no interaction with other main QTL(s). The reason is that WinQTLCart is allow existing of main QTL that has no (very little) effect but has strong interaction effect in score statistics test situation.

QTL Interactions - Test each QTL interaction to see it's significant or not.

Clicking **Start** returns you to a slightly modified MIM model, where the operation will continue until the result is obtained.

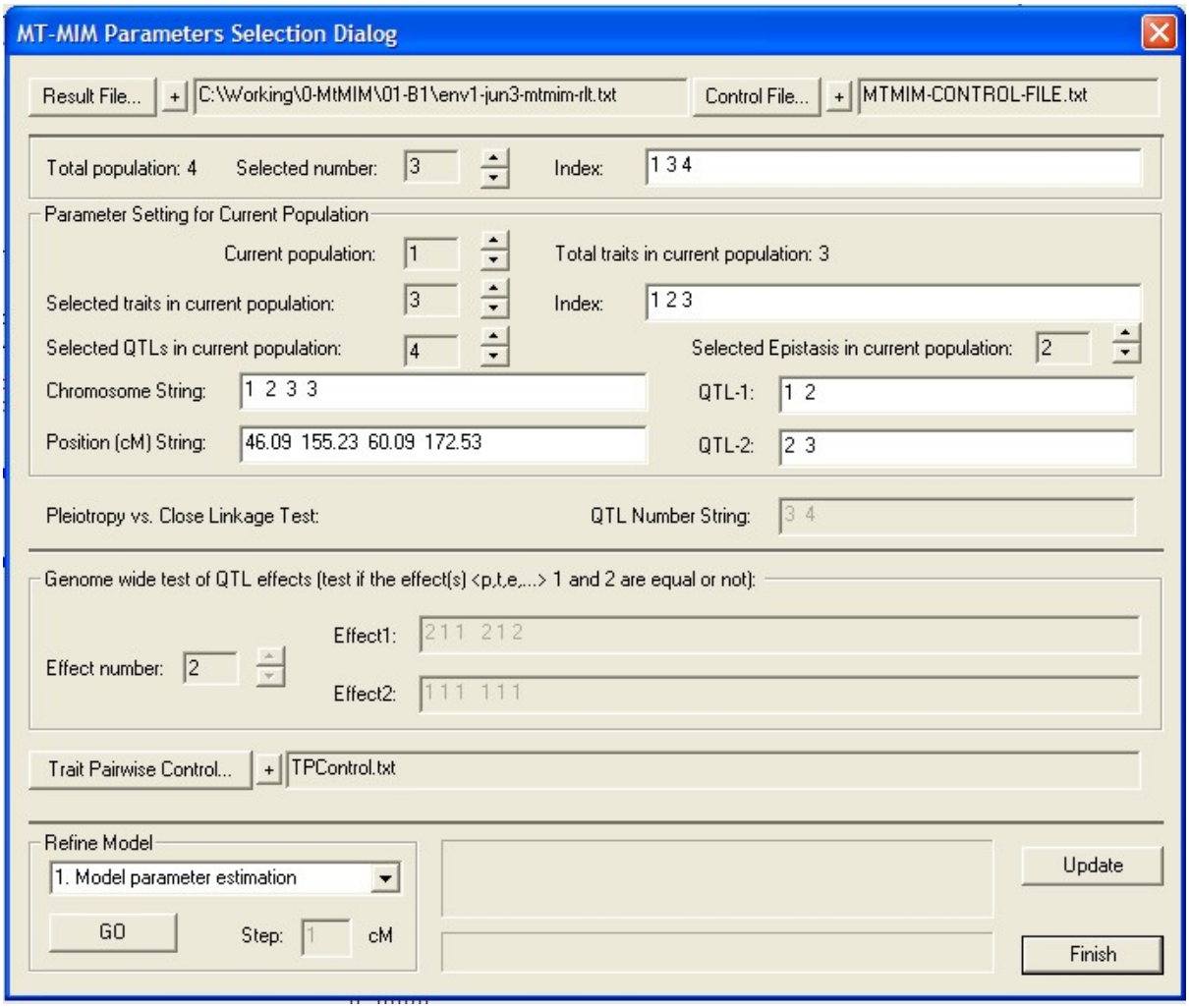
Note *To create a MIM results file in .QRT format, select the MIM model summary option.*

Multiple-trait MIM

Multiple QTL Mapping (MIM) for multiple-trait or multiple-environment in multiple-population .

About the Mt-MIM form

From the Source Data form, open a source data file and select Multiple Interval Mapping as the analysis method. If there is more than one trait in the dataset, the Select Trait for MIM Analysis dialog appears. Click Mt-MIM button to open MT-MIM Parameters and Analysis Dialog.



Result File. Click **Result File** button change the result filename and click **+** button to open the result

file with Windows notepad.

Control File. Click **Control File** button to change the control filename and click **+** button to open the control file with Windows notepad. The control file includes all initial parameters setting in the dialog, detail please to see Mt-MIM Control File 74.

Population Group. Total population refers to the total population number in the source data. **Selected number** indicates how many populations will be in the QTL mapping model. To use **Index** edit box to indicate the index of selected populations comparing to all populations in original source data.

Parameter setting for current population Group. Click the spin dial beside the **Current population** value to set current population number first. Then to fill other parameters in the rest of group.
Note: The current population number depends on selected population number only. For example, if the selected population number is 3, then the current population will be 1, 2, and 3 despite where these population is located in source data file.
For each current population:
Total traits in current population. The number refers to the total traits (or environments) in the source data.
Selected traits in current population. The number indicates that how many traits will be in the QTL mapping model.
index. The number string indicates the index of selected traits.
Selected QTLs in current population. Click the spin dial to set the QTL number for current population.
Chromosome String. Chromosome number for QTL1, QTL2, ...
Position (cM) String. Positions for QTL1, QTL2, ...
Selected Epistasis in current population. Click the spin dial to set the QTL interaction terms for current population.
QTL-1. QTL number list. They are the first QTL number between two QTL interaction.
QTL-2. QTL number list. They are the second QTL number between two QTL interaction.

Pleiotropy vs. Close Linkage Test Group. The purpose of the test is to see that two or more QTL is the same or not statistically. The QTL number list is in **QTL Number String** edit box. Please notice that you can do the test for one or more population all together.

Genome wide test of QTL effects. The purpose of the test is to see that two group of QTL effects (QTL main effect or epistatic effect) are the same or not statistically. **Effect number** is the QTL effect numbers in a effect group. **Effect1** and **Effect2** indicate group 1 effects and group 2 effect respectively. Each QTL effect includes three number that are population number, trait number and QTL effect number.

Trait Pair wise Test. Click **Trait Pair wise Test** button to open the control file and click **+** button to open the control file with Windows notepad.

Refine Model Group. Click the pull-down menu to select a function (please to see Mt-MIM Functions 75) and click **Go** button to start calculation. Modifying **Step** Edit Box to indicate the walk step in cM while searching through chromosomes.

Update. Click the button after changing parameters setting.

Finish. Click the button to finish Mt-MIM analysis and the software will remind you to save the parameters setting into a new control file.

Mt-MIM Control File

The following is a example of control file used for source data file **env1-jun3.mcd**, env*2*-jun3.mcd, env*3*-jun3.mcd, and env4-jun3.mcd. There are totally 4 populations in the source data.

-populations 3 1 3 4	line 1
#population-1#	line 2
-traits 3 1 2 3	line 3
-qtls 4 1 46.09 2 155.23 3 60.09 3 172.53	line 4
-epis 2 1 2 2 3	line 5
#population-2#	line 6
-traits 2 2 3	line 7
-qtls 3 1 46.09 2 155.23 3 60.09	line 8
-epis 1 1 3	line 9
#population-3#	line 10
-traits 2 1 3	line 11
-qtls 3 1 46.09 3 60.09 3 172.53	line 12
-epis 0	line 13
-pvsltest 2	line 14
-p 1 2 3 4	line 15
-p 3 2 2 3	line 16
-gqtltest 2	line 17
-str1 1 1 1 1 1 2	line 18
-str2 2 1 1 2 1 2	line 19
-end	line 20

Token **-populations.** (line 1) The first number indicates how many populations will be in the QTL mapping model. The rest number(s) is/are selected population(s). (using index of selected populations comparing to all populations in original source data.)

Token **#population.** (line 2, 6, 10) This is the star line of parameters setting for each selected population.

Token **-trait.** (line 3, 7, 11) The first number is the total trait number for current population and the rest is/ are selected trait index.

Token **-qtls**. (line 4, 8, 12) The first number is the total QTL number and the rest are chromosome number and position in cM for each QTL.

Token **-epis.** (line 5, 9, 13) The first number is the total QTL interaction number. Then, for each QTL epistasis, there are two numbers that indicate which two QTLs that are interacted.

Token **-pvsltest.** (line 14) Pleiotropy vs. close linkage test. The number after the token is how many populations that are in the test.

Token **-p.** (line 15, 16) One line started with **-p** token for each population of pleiotropy vs. close linkage test. The first number indicates which population. Then the total QTL number to be test and followed by the QTL indexes.

For example. -p 1 2 3 4, Population 1, to test QTL 3 and QTL 4 are actually one QTL or are two different QTLs.

Token **-gqtltest.** (line 17) To test two sets of QTL effects (see token **-str1** and **-str2**) have the same effects or not. The number followed by the token is the QTL effect number of each set.

Token **-str1** and **-str2.** (line 18, 19) Every three numbers (population, trait, and QTL) indicate a QTL effect.

For example.

-gqtltest 2
-str1 1 1 1 1 1 2
-str2 2 1 1 2 1 2

This is to test the following assumption: population 1, trait 1, QTL effect 1 equal to population 2, trait 1, QTL effect 1 as well as population 1, trait 1, QTL effect 2 equal to population 2, trait 1, QTL effect 2. or not.

Token **-end.** (line 20) End of Mt-MIM Control File.

Mt-MIM Functions

In the **Refine Model Group,** you can select one of following functions from the pull-down menu.

1. Model parameter estimation

To do the parameters estimation for current model. The likelihood value is displayed in information window on the bottom and the detailed result is in the result file. You can compare the likelihood values between two QTL models (likelihood test) to do certain decision, such as the new QTL is significant or not, The two QTL is the same or not etc.

2. Test all QTL effects

To test the value is zero or not for each of the QTL effects (additive, dominant, and epistasis).

3. Optimize QTL positions

To go through whole genome (you can set the walk speed, such 1.0 cM), find best position for each QTL.

4. Search main QTL(s)

If you do not know the new QTL's chromosome and position, then to assign value -1 to the chromosome string and WinQTLCart will search the best chromosome number and position for you automatically.

5. Search epistasis interaction(s)

If you want to find the best QTL interaction, then assign value -1 to QTL-1 and QTL-2 and WinQTLCart will find out the best QTL number 1 and QTL number 2 for you automatically.

6. Graphic and summary result

To obtain the graph result file and text summary file.

7. Test Pleiotropy in current population

To do the pleiotropy vs. close linkage test according to above parameter setting.

8. Genome wide test of QTL effects

   To do the QTL effect(s) equality test according to above parameter setting.

9. Trait pairwise co-relation in current population

   To calculate QTL co-relation between pairs of traits according to a control file (TPControl.txt).

Example of TPControl file:

```
#begin
1 gene_563 gene_2694 3 3 54.6 15 52.5 13 288.1
2 gene_563 gene_4898 2 3 54.6 15 52.5
3 gene_563 gene_5323 2  3 54.6 15 52.5
#end
```

The control file start with line of #begin and end with line of #end. For each line between is the information for a pair of traits. The first number is the numbering, and second and third are the marker labels for the first and second trait. After that is the total QTL number follow by chromosome number and position for each QTL.

Bayesian Interval Mapping

WinQTLCart's Bayesian interval mapping (BIM) module is an implementation of the command-line BIM library, provided courtesy of the R Project for Statistical Computing.

What it is
Bayesian interval mapping library R/bim provides Bayesian analysis of multiple quantitative trait loci models. This includes posterior estimates of the number and location of QTL and of their effects.

Bayesian interval mapping for controlled experiment provides a nice complement to the classical analysis for mapping QTLs. It is recommended that the standard IM, CIM, MIM, etc. analyses be run first. The Bmapqtl implements Bayesian analysis for either a fixed or random number of QTLs. (WinQTLCart's default is random.)

The program generates a random sample from the joint posterior of QTL and effects. Note that the BIM estimates should generally agree with those of MIM, and should be similar to those from CIM.

BIM allows one to look deeper at the properties of those estimates, such as how effects estimates are related to loci estimates. It also provides handy tools to explore research questions, such as the posterior chance of multiple QTL in an interval.

How to use
The defaults are quite robust, and it is recommended you use them during your first BIM runs.

For more information on the BIM parameters
Please refer to the R implementation, particularly the function bmapqtl.options(). The best way to get library(bim) is to already have R 1.9.0 installed on your PC.

1. If you don't have R 1.9.0 installed on your computer, go to http://cran.r-project.org/
2. Download the precompiled binary distribution of R (Linux or Windows is preferred.) Follow

instructions for installation and run R.

3. While connected to the Internet, select R's Packages menu and select Install packages from Bioconductor.
4. Scroll down to the "bim" package and click on it.
5. Use the R Help to get HTML Help.
6. Select Packages and click BIM from the list. There is an overview document but the options are explained in detail in bmapqtl.options.

Note *Pre-1.9.0 releases of R can get the bmapqtl package from the CRAN website cited above. Bioconductor is a companion project to CRAN focused on biological applications. Brian*

A quick summary of BIM implementation in a previous version of QTL Cartographer can be found at http://www.cs.wisc.edu/~yandell/qtl/software/Bmapqtl/Bmapqtl.pdf.

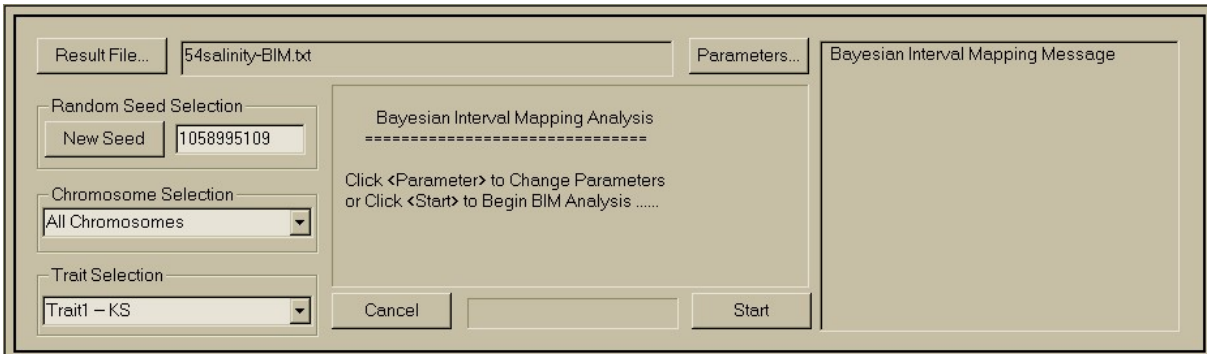
High-level workflow

The first few times you run this analysis, go with the WinQTLCart default values for the form's parameters. The defaults provide the best all-around parameter settings, especially for initial analysis sessions.

1. Select the BIM analysis method.
2. Select the chromosome(s) and trait(s) you want to analyze.
3. Click New Seed to select a new random seed.
4. Click Parameters… and set the BIM parameters of interest to you.
5. Click Start to begin the analysis.

Running Bayesian interval mapping analysis

1. Open a source data file into the WinQTLCart main window.
2. Select Method>Bayesian Interval Mapping. WinQTLCart displays the BIM analysis controls in the form pane.



3. Click Result File… to name the .QRT file you want to create and to specify its location.
4. Click New Seed to generate a new random seed number.
5. Select one or all chromosomes from the Chromosome Selection drop down to include in the analysis.
6. Select one or all traits from the Trait Selection drop down to include in the analysis.
7. Click the Parameters… button to display the Set BIM Parameters dialog.
   For more information on each of these parameters, please refer to the R bmapqtl documentation cited in the topic, "Bayesian Interval Mapping 76".
8. Click Start to begin QTL mapping analysis.

Multiple-trait analysis

Most of the analysis methods in WinQTLCart work on traits one by one. The multiple trait analysis lets you pick and choose the traits you want to work with jointly. This allows you to perhaps derive and correlate structures from among many separate traits.

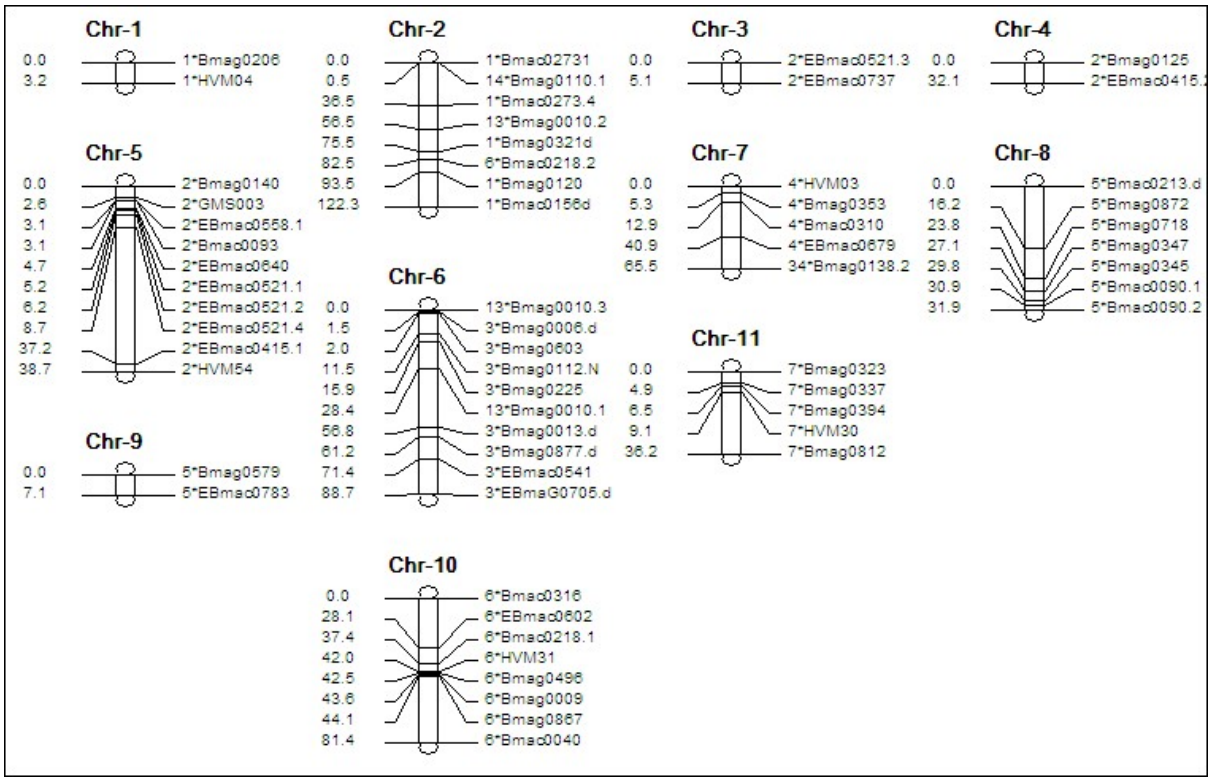
1. Open a source data file in WinQTLCart. If the data contains multiple traits, WinQTLCart enables the Method menu's Multiple Traits Analysis command.
2. Select Method>Multiple Traits Analysis. The Multiple Traits Analysis dialog appears. WinQTLCart disables portions of the dialog box that cannot support analysis of the selected data file.
   - B1 crosstype has additive effects and enables only method selection
   - F2 crosstype has dominant effects and enables hypothesis test selection (open the WinQTLCart sample file "cod.mcd" for an example)
3. Select the analysis method you want to use, IM or CIM.
4. Select the traits you want to use in the analysis. Click the Trait List button to see available traits for selection. In the Trait selection text box, enter the traits you want to use, separated by commas.
   So, if you want to use traits 1, 4, and 5, you would enter "1, 4, 5" (without the quote marks).
5. Click OK. The form pane takes on the standard IM/CIM look and function, but the countdown pane prefixes the method with "Multiple Traits."
6. For permutation procedure, check Independent check box to randomize each trait independently. Otherwise to keep the co-relationship among traits when doing randomization.

Refer to the IM 58 and CIM 60 procedures in this manual for more information on using the form.

Drawing a chromosome tree

You may need to use chromosome graphics when it's time to write an article on your research. WinQTLCart displays all the source data file's chromosomes, including each chromosome's markers and intervals, in a single display window that you can copy to the Windows clipboard.

From the Main window, with a source data file loaded, select Tools>DrawChrom to show the Chromosome Graphic Display window.



This sample graphic shows you the chromosome names, the markers on the chromosomes, and their distances.

Chromosome Graphic Display menus

File menu

Command	Function
Copy to Clipboard	Copies the graphs to the Windows clipboard.
Print Graphic	Print the graphic to a selected printer.
Exit	Closes the window. If you have unsaved data, you'll be prompted to save it.

View menu

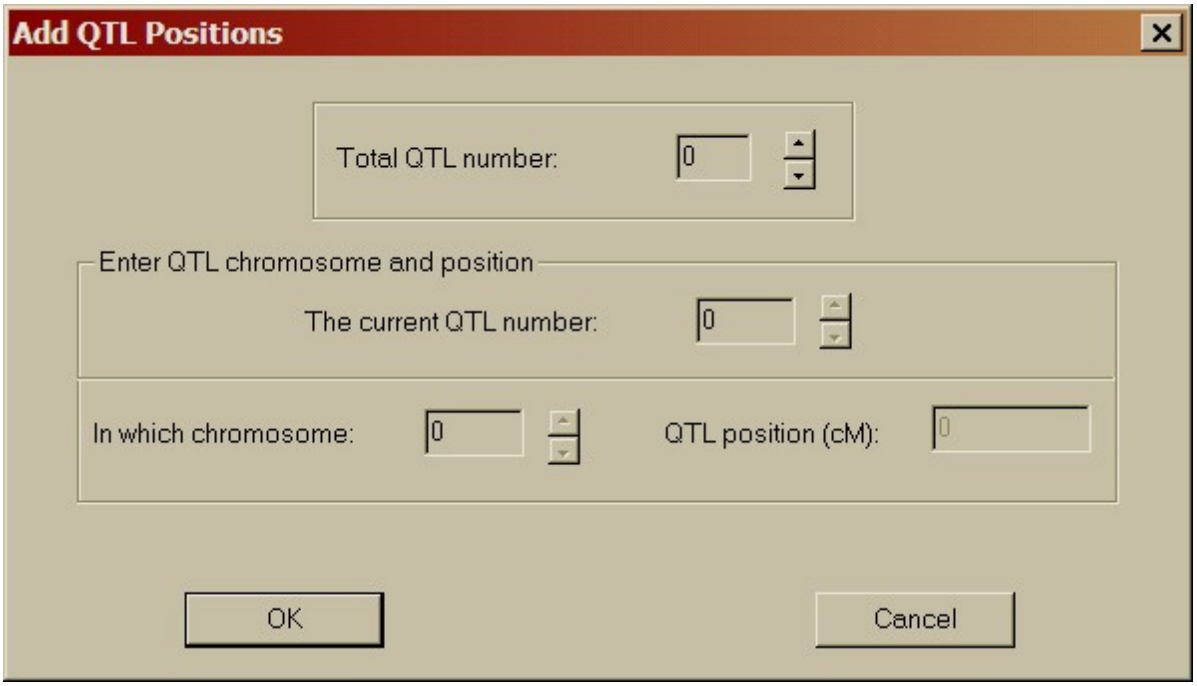
Command	Function
Proportion of Marker Number	Show length of chromosome graph in proportion of marker number
Proportion of Chromosome Len	Show length of chromosome graph in proportion of chromosome length in cM
Next Page >>	If there are lots of chromosomes, displays the next group of graphs
First Page	If there are more than one screen of chromosomes, return to the first page.
Add QTL positions…	Mark a QTL position on the chromosome. See Adding QTL positions to the chromosome graphics 80 for more information.

Setting menu

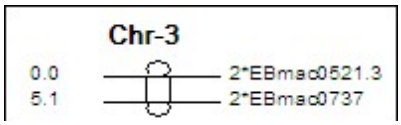
Command	Function
Select Chromosomes…	Select the chromosomes you want displayed in the graphic.
Show Chromosome Name	Toggle between showing the chromosome name or its label
Font Size >>	Increase font size for graph
Font Size <<	Decrease font size for graph
Space Between >>	Increase the space between markers (graph gets longer)
Space Between <<	Decrease the space between markers (graph gets shorter)
Chromosome Name>>	Go to the next chromosome in the series
Chromosome Name<<	Go to the previous chromosome in the series
Column Number >>	Increase number of graphics displayed in a column
Column Number <<	Decrease number of graphics displayed in a column

Adding QTL positions to the chromosome graphics

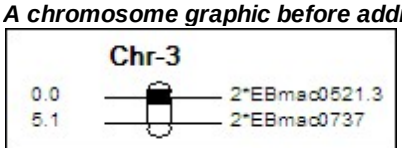
At the Chromosome Graphic Display window, select View>Add QTL Positions to display the Add QTL Positions dialog box.



1. Click the spin dial in the Total QTL number dialog to select the number of QTLs you want to add to a graphic.
2. If you're placing more than one QTL in the Current QTL number box, use the spin dial to select which QTL you want to place.
3. For the In which chromosome field, use the spin dial to select the chromosome that will hold the QTL.
4. In the QTL position (cM) field, enter the position of the current QTL, in centiMorgans.
5. Click OK. WinQTLCart places a band over the chromosome graphic indicating the QTL location.



A chromosome graphic before adding QTL positions...



...and after adding QTL positions

Tutorials

Import data files

Create source data file (*.mcd) by using different formats of data file(s).

Import data - INP format

1. Start WinQTLCart and select menu item **File>Import** or click 

.
2. Select **QTL cartographer INP format** and click **Next** button.
3. Click button **Map File** to select file "**wqcart-map.inp**".
4. Click button **Cross Data** to select file "**wqcart-cro.inp**".
5. Enter file name "**wqcart_inp_In**" in edit box and click button **Finish**.

Using Emap function

1. Start WinQTLCart and select menu item **File>Import** or click 

.
2. Select **QTL cartographer INP format** and click **Next** button.
3. Check "Infer map information from cross data file".
4. Click button **Cross Data** to select file "**Csamp0.inp**".
5. Enter file name "**Csamp0_inp_In**" in edit box and click button **Finish**.

Import data - OUT format

1. Start WinQTLCart and select menu item **File>Import** or click 

.
2. Select **QTL cartographer OUT format** and click **Next** button.
3. Click button **Map File** to select file "**wqcart.map**".
4. Click button **Cross Data** to select file "**wqcart.cro**".
5. Enter file name "**wqcart_out_In**" in edit box and click button **Finish**.

Import data - MapMaker format

1. Start WinQTLCart and select menu item **File>Import** or click  .
2. Select **Mapmaker/QTL format** and click **Next** button.
3. Click button **Map File** to select file "**mapmaker-samp.map**".
4. Click button **Cross Data** to select file "**mapmaker-samp**".
5. Enter file name "**mapmaker-samp_In**" in edit box and click button **Finish**.

Import data - Excel format

1. Start WinQTLCart and select menu item **File>Import** or click  .
2. Select **Microsoft Excel format** and click **Next** button.
3. Click button **Excel File** to select file "**wqcart-samp.xls**".
4. Enter file name "**wqcart-samp_xls_In**" in edit box and click button **Finish**.

Import data - CSV format

1. Start WinQTLCart and select menu item **File>Import** or click  .
2. Select **Microsoft CSV format** and click **Next** button.
3. Click button **CSV File** to select file "**NSimuB1-01-OnePop.csv**".
4. Enter file name "**NSimuB1-01-OnePop_in**" in edit box and click button **Finish**.

Simulation source data file

1. Start WinQTLCart and select menu item **File>Simulation** or click  .
2. In step 1/3, set parameters as:

Sample size:	200
Trait mean:	15.85
Random seed:	any
Map function:	Haldane
Cross type:	B1
Translation table:	AA>2, Aa>1, A>*
Total chromosome number:	3

3. Set **The current chromosome** as 1, **Markers for chromosome 1** as 15, **Average space between marker** as 9, **Variations of the marker position** as 10%. Click button **Set as default for all chromosomes** to set parameter for other chromosome. Click button **Next** to step 2/3 dialog window.
4. In step 2/3, set parameters as:

QTL numbers:	7
Heritability:	0.82
Additive effect:	Effects direction>Same Effects distribution>Normal
Epistatic effect:	**None** by uncheck the check box
Filename:	"Nsimu-01"

Click button **OK** to step 3/3 dialog window.

5. In step 3/3, Click button **Adjustment** to adjust all parameters. Click button **Save** to save the parameter setting to file "Nsimu-01.qpe". Click button **OK** to finish.

Create new source data file

1. Start WinQTLCart and select menu item **File>New** or click 

.
2. Set parameters in step 1 of 2 as:

Chromosome number:	2
Trait number:	2
Other trait number:	1
Individual number:	125
Symbol of missing trait:	.
Crossing type:	B1
File stem name:	Test
AA	2
Aa	1
Aa	*

3. Click **OK** to step 2 of 2.
4. Click button **Notepad** to open file "**Text.txt**" in default directory (C:\NCSU\WinQTLCart).
5. In **Notepad**, select chromosome label (**CHROM-1, CHROM-2**) and copy to Windows clipboard. Minimize **Notepad**.
6. Back to Create New Source File – Step 2 0f 2. Click button **Paste** to show chromosome labels in Edit Window. Select **Chromosome Labels** in Data Type Selection. Click button **Send Data** to input chromosome labels.
7. Select and copy **Marker Number** in **Notepad**. Click button **Paste**, click **Marker numbers** in Data Type Selection and click button **Send Data** to input marker numbers.
8. Select and copy **Marker Label and Position** in **Notepad**. Click button **Paste**, Select **Marker positions** in Data Type Selection and click button **Send Data**. Answer "**Y**" in the dialog to input marker labels and positions.
9. Select and copy **Marker Genotype** and **paste**. Click **Marker genotypes** and click button **Send Data** . Answer "**Y**" to input marker genotype data.
10. Select, copy and Paste **Trait Value**. Select **Trait Values** and click button **Send Data**. Answer "**Y**" to input trait values.
11. Select, copy and Paste **Other Trait Value**. Select **OTrait** Values and click button **Send Data.** Answer "**Y**" to input other trait values.
12. Click button **OK** to create source data file (Test.mcd).

Single marker analysis

1. Select Menu Item **File>Open** or click 

to open source data file "**wqcart-samp0.mcd**".
2. Select Menu Item **Method>Single Marker Analysis** to open the Form of single analysis.
3. Use the **poll-down menu** to select a trait. Click button **Result** to show information of single marker analysis or statistical summary.
4. Click button **Graph File** to produce result file and show in graph dialog.
5. Click button **OK** to finish single marker analysis.

Interval mapping

1. Select Menu Item **File**>**Open** or click  to open source data file "mouse.mcd".
2. Select Menu Item **Method**>**Interval Mapping** or click  to open the Form of IM analysis.
3. Select **By Permutation** and click button **OK** to start threshold value calculation.
4. Click button **Start** to start IM analysis.
5. Click "**Y**" to show QTL summary in Text Window.

Composite interval mapping

1. Select Menu Item **File>Open** or click  to open source data file "**wqcart-samp0.mcd**". You can just click the tree item if the file has already been opened.
2. Select Menu Item **Method>Composite Interval Mapping** or click  to open the Form of CIM.
3. Click button **Control** to set the parameter. Set **Control marker number** as 8, **Window size** as 15.0, **Other trait** as 1,2 and click button **OK** to back.
4. Select **All traits** in trait selection pull-down menu.
5. Click button **Start** to begin CIM analysis.

Multiple-trait analysis

1. Select Menu Item **File>Open** or click  to open source data file "**wqcart-samp0.mcd**".
2. Select Menu Item **Method>Multiple Traits Analysis**.
3. Select **IM Method** and input **1,3** in edit box for Multiple Traits Setting Dialog.
4. Click button **Start** to begin multiple traits analysis.

Multiple Interval Mapping

1. Select Menu Item **File>Open** or click  to open source data file "**wqcart-samp0.mcd**".
2. Select Menu Item **Method>Multiple Interval Mapping** or click  and select trait **MECIAIA** to enter the Form of MIM.
3. Click button **New Model**, click "**Y**", and click button **OK** to obtain an initial MIM model.
4. Click button **Refine Model**. Select **Optimizing position** and click button **OK**.
5. Click button **Start** to start QTL position optimization procedure.
6. Click button **Save Model** to save the QTL model.
7. Click button **Refine Model**. Select **Searching for new QTLs** and click button **OK**.
8. Select **Search for QTLs** and click button **Start** for searching for more QTL.
9. Click button **Refine Model** and optimize QTL position again.
10. Click button **Test All** to see LR values for each QTL in Text window.
11. Click button **Save Model** to save the QTL model again. You may change filename if you want to keep the previous QTL model.

12. Click button **Refine Model**. Select **Searching for new QTLs** and click button **OK**. Select **Search for Epistasis** and click button **Start** for searching for QTL interact (epistasis). Answer "**Y**" to add the epistatic effect.
13. click button **Refine Model**. Select **MIM Model Summary** and click button **OK**.
14. Select **Graphic Result File** and click **Start** to produce result file and show it in graph dialog.
15. click button **Refine Model**. Select **MIM Model Summary** and click button **OK**. Select **Model Summary File** and click **Start** to produce MIM summary information.
16. In tree pane, you can click to show information of breed value, QTL information, R2 partition, and variance and co-variance table.
17. Click button **Finish** and button **Save** to finish the MIM session.

Bayesian interval mapping

1. Select Menu Item **File>Open** or click 

to open source data file "**wqcart-samp0.mcd**".
2. Select Menu Item **Method>Bayesian Interval Mapping** to enter the Form of BIM.
3. You can click button **Parameter** to set the BIM parameters.
4. Click button **Start** to begin CIM analysis.

Result manipulation

1. Start WinQTLCart and select menu item **View>Visualize Result** or click 

to open result file "**wqcart-samp0-C.qrt**".
2. Use menu item **Chrom** to select and show one chromosome, all chromosomes, and any number of chromosomes with different order.
3. Use menu item **Traits** to select and show any number of traits.
4. Select menu item **Tools>Display one page format** or click 

to show graph in different format.
5. Select menu item **Setting>set display parameters** or click 
to adjust parameter of graph.
6. Select menu item **Tools>Show QTL information** or click 
to obtain the QTL summary information from the graph or open a QTL summary file (*.qtl).
7. you can trace on graph by selecting menu item **Setting>Trace coordinate in graph** or click 
. Double click to show the coordinate numbers in graph.
8. You can combine two graphs into one by selecting menu item **File>Add QTL result file**.

Technical notes

Troubleshooting

1. Errors even to run Single Marker Analysis

WinQTLCart has problem to deal with complex folder and file name. To avoid this problem, please try put your MCD file in a simple folder such \NCSU\Work1\ and only letter and digital are in file name.

2. Why my trait values are truncated into integers

Correct Windows setting by Control Panel - Regional and Language - Regional Options. Make sure that the Number format is 123,456,789.00

3. WinQTLCart cannot import Map information from selected file

The Map file you've selected doesn't have a format that WinQTLCart recognizes. Open your file in Notepad and compare its format to one of the sample MAP or CRO files in the WinQTLCart directory.

Problems also occur when a map file is selected as the cross file, or a cross file is selected as the map file.

Related topics
Compatible programs and formats 1

4. Invalid file or wrong format messages

You may see WinQTLCart error messages complaining that a file you're opening or importing is invalid, is in the wrong format, cannot be recognized, and so on.

The file's data may not be in a format WinQTLCart recognizes or the file may have been formatted incorrectly or its extension might have changed. Open up the file in Notepad and compare it to a sample file of the same extension in the WinQTLCart directory.

Related topics
Compatible programs and formats 1

5. Failures when I try to creat MCD file from text files

The easiest way to create a MCD file is by using method of Import - CSV format. You can use the sample file "NSimuB1-01-OnePop.csv" as a model to create your own CSV data file.

Technical Support

Ensure you have the latest release

1. Select Help>About WinQTLCart and look at the software release date. Click on the Update Site link to go to http://statgen.ncsu.edu/qtlcart/WQTLCart.htm, the WinQTLCart release page.
2. If the date on the page is more recent, then scroll down to the Downloads section and get the latest copy.
3. Upgrading to a new version of WinQTLCart does not overwrite your working files. However, the upgrade will replace the sample files that are part of the WinQTLCart distribution.

If you have questions

Send an email to WinQTLCart Tech Support at <mailto:shchwang@statgen.ncsu.edu?subject=WqtlcartUser>. Please include the following details in your mail:

- WinQTLCart version you're using
- Describe the task you're trying to accomplish, what you expected to see, and what WinQTLCart actually did
- Please describe the error message you received (if any)

Credits & acknowledgements

Windows QTL Cartographer was created by Shengchu Wang, C.J. Basten, and Z.-B. Zeng.

Thanks to P. Gaffney, Sara Via, Ann Stapleton, Heike Kross, and Janice Cuthbert.

This file was originally written by Michael E. Brown and was produced using EC Software's Help & Manual. Site: http://www.helpandmanual.com

Index