User Manual for

QTL.gCIMapping.GUI

QTL genome-wide Composite Interval Mapping GUI

(version 2.1.1)

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Disclaimer: While extensive testing has been performed by Yuan-Ming Zhang's Lab (Statistical Genomics Lab) at Huazhong Agricultural University, the results are, in general, reliable, correct or appropriate. However, results are not guaranteed for any specific datasets. We strongly recommend that users validate the GCIM results with other software packages, such as **Windows QTL Cartographer V2.5_011** (https://brcwebportal.cos.ncsu.edu/qtlcart/WQTLCart.htm) and **QTL IciMapping V4.1** (http://www.isbreeding.net/software/?type=detail&id=18).

Download website:
https://cran.r-project.org/web/packages/QTL.gCIMapping.GUI/index.html

References

1 Wang Shi-Bo, Wen Yang-Jun, Ren Wen-Long, Ni Yuan-Li, Zhang Jin, Feng Jian-Ying, Zhang Yuan-Ming*. Mapping small-effect and linked quantitative trait loci for complex traits in backcross or DH populations via a multi-locus GWAS methodology. ***Scientific Reports*** 2016, 6: 29951.

2 Wen Yang-Jun, Zhang Ya-Wen, Zhang Jin, Feng Jian-Ying, Jim M. Dunwell, Zhang Yuan-Ming*. An efficient multi-locus mixed model framework for the detection of small and linked QTLs in F_2. ***Briefings in Bioinformatics*** 2019, 20(5): 1913-1924.

3 Zhang Ya-Wen, Wen Yang-Jun, Jim M. Dunwell, Zhang Yuan-Ming*. QTL.gCIMapping.GUI v2.0: An R software for detecting small-effect and linked QTLs for quantitative traits in bi-parental segregation populations. ***Computational and Structural Biotechnology Journal*** 2020, 18: 59-65.

4 Wen Yang-Jun, Zhang Ya-Wen, Zhang Jin, Feng Jian-Ying, Zhang Yuan-Ming*. The improved FASTmrEMMA and GCIM algorithms for genome-wide association and linkage studies in large mapping populations. ***The Crop Journal*** 2020, 8(5): 723-732.

Quantitative Trait Loci

Genome-wide Composite Interval Mapping

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INTRODUCTION

1.1 The function of the GCIM software

QTL.gCIMapping.GUI v2.1.1 (**QTL G**enome-wide **C**omposite **I**nterval **M**apping **G**raphical **U**ser **I**nterface) is an R package for multi-QTL mapping of quantitative traits in bi-parental segregation populations, including backcross (BC), doubled haploid (DH) lines, recombinant inbred lines (RIL), F_2, and immortalized F_2 (IF_2). QTL.gCIMapping.GUI v2.1.1 works well on the R environment on Windows, Linux (desktop) and MacOS.

1.2 Getting started

The software package QTL.gCIMapping.GUI v2.1.1 can be freely downloaded from https://cran.r-project.org/web/packages/QTL.gCIMapping.GUI/index.html, or BioCode (https://bigd.big.ac.cn/biocode/tools/BT007078) or request from the maintainer, Dr Yuan-Ming Zhang at Crop Information Center, College of Plant Science & Technology, Huazhong Agricultural University (soyzhang@mail.hzau.edu.cn).

1.2.1 One-Click online installation

On R environment and network connection, the command
install.packages(pkgs="QTL.gCIMapping.GUI")
is used to directly install the software package QTL.gCIMapping.GUI v2.1.1.

1.2.2 Step-by-step offline installation

1.2.2.1 Install the add-on packages

First, users download thirty-nine R packages, including
"crayon","data.table","digest","doParallel","fastmap","foreach","glmnet","glue","htmltools","httpuv","iterators","jsonlite","later","magrittr","mime","openxlsx","promises","QTL.gCIMapping","qtl","R6","Rcpp","rlang","shape","shiny","stringi","stringr","xtable","zip"
from CRAN, github (https://github.com/), or google search.

On the R environment, then, users select all the 28 packages and install them offline.

1.2.2.2 Install QTL.gCIMapping.GUI v2.1.1

On R GUI environment, users first select "Packages"—"Install package(s) from local files…", then find the software package QTL.gCIMapping.GUI v2.1.1 on user's desktop computer or mobile device, and launch QTL.gCIMapping.GUI v2.1.1.

1.2.3 Run QTL.gCIMapping.GUI v2.1.1

Once the software package QTL.gCIMapping.GUI v2.1.1 is installed, users may run it using two commands:

library(QTL.gCIMapping.GUI)

QTL.gCIMapping.GUI()

If users re-use the software QTL.gCIMapping.GUI v2.1.1, users use the above two commands as well.

User Manual file Users can decompress the QTL.gCIMapping.GUI package and find the User Manual file (name: **Instruction.pdf**) in the folder of "…/QTL.gCIMapping.GUI/inst/doc".

2. Dataset format

GCIM format for Dataset The first three columns, named "**marker**", "**chr**" and "**pos**", stand for marker name, chromosome and marker position (cM) on the chromosome, respectively. Among the remaining columns, each column lists all the genotypes of one individual or line, while the first row shows the name of the individual or line. For the genotypes of each marker, the coding criteria are shown as Table 1.

Table 1. Coding criteria for GCIM format

Marker genotype	Code	Meaning
AA	A	Homozygous genotype (P_1)
Aa	H	Heterozygous genotype (F_1)
aa	B	Homozygous genotype (P_2)
Not AA (Aa + aa)	C	Dominance to P_2
Not aa (AA + Aa)	D	Dominance to P_1
Missing	-	Missing or unclear genotype

The genotypic, phenotypic and covariate datasets are located on the upper, middle, lower sections, and each covariate or trait is presented on one row. On each row, the

first column is empty followed by "**trait1**", "real trait name", and "phenotypic values for all the individuals or lines". If there are multiple traits, these traits occupy multiple lines. **"NA" indicates the missing or unknown phenotypes.** If there are covariates, the content lies below the trait dataset. The format is seen in Table 2. If there is no covariate, users should delete the last row in Table 2.

Table 2. The GCIM format of the dataset

marker	chr	pos	DH6-10	DH6-101	DH6-102
RGA3(1)	1	0	B	-	B
wPt-6358	1	3.034	B	-	-
Hplc2	1	8.8291	A	A	B
wPt-9752	1	10.1452	A	-	-
abc156a	1	41.3408	A	A	B
⋮	⋮	⋮	⋮	⋮	⋮
gwm437	21	162.5218	A	B	-
gwm121	21	180.2878	A	B	-
wmc157	21	197.9196	A	B	A
*stm1actc	21	200.4216	-	-	-
	trait1	T19	75.33	105	96.33
	trait2	T191	74	105.68	97.16
	trait3	T192	75.37	104.67	95.55
	Covar1	CovarName	A	B	B

The format of ICIM dataset If users have the QTL IciMapping dataset, these files are also available in our software. Details can be found in the folder of "…/QTL.gCIMapping.GUI/inst/extdata", i.e., WheatDH_QTLIciMapping_Format.xlsx.

The format of WinQTLCart dataset If users have the WinQTLCart dataset, its file is also available in our software. Details can be found in the folder of "…/QTL.gCIMapping.GUI/inst/extdata", i.e., env1-jun3_WinQTLCart_Format.mcd.

The format of ICIM covariate dataset If users use the ICIM dataset and there are covariates, users need to input a covariate file. In the file, the first column indicates

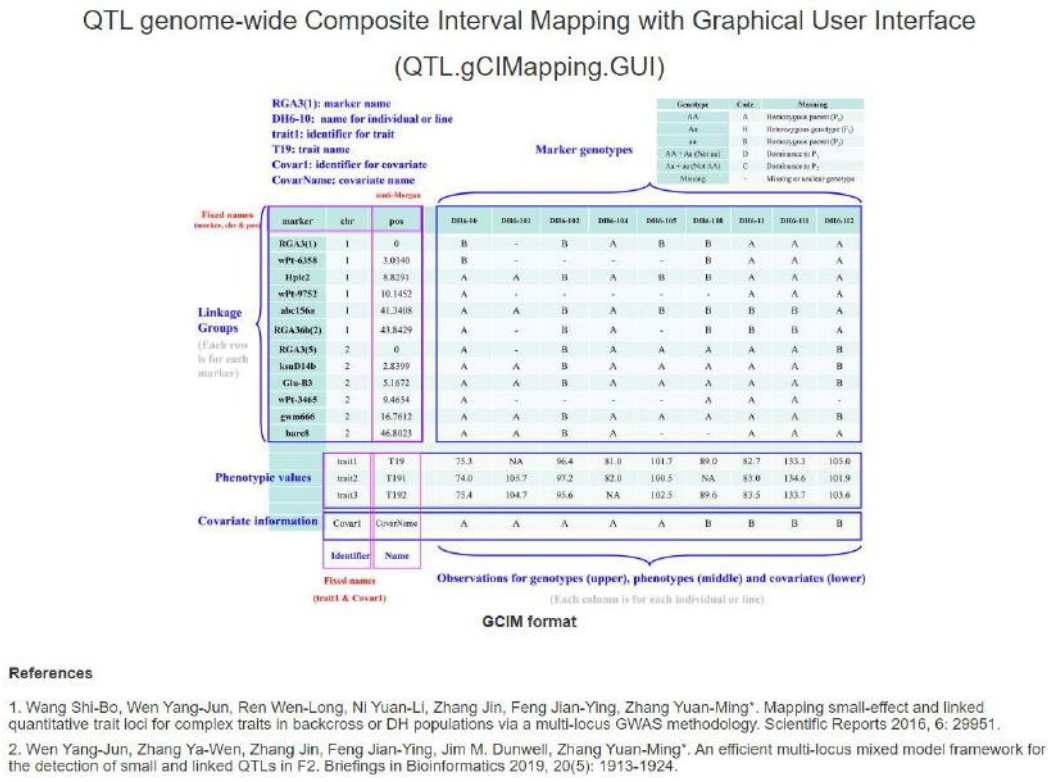
individual name and the second column is the covariate information (Table 3). In Table 3, the covariate values are indicated by such as A, B and C.

Table 3. The covariate file format

Individual ID	Covariate
DH6-10	A
DH6-101	A
DH6-102	A
DH6-104	A
DH6-164	B
DH6-165	B
DH6-166	B
DH6-170	B
DH7-124	C
DH7-125	C

3 Operation process

3.1 The graphical interface of QTL.gCIMapping.GUI v2.1.1



References

1. Wang Shi-Bo, Wen Yang-Jun, Ren Wen-Long, Ni Yuan-Li, Zhang Jin, Feng Jian-Ying, Zhang Yuan-Ming*. Mapping small-effect and linked quantitative trait loci for complex traits in backcross or DH populations via a multi-locus GWAS methodology. Scientific Reports 2016, 6: 29951.

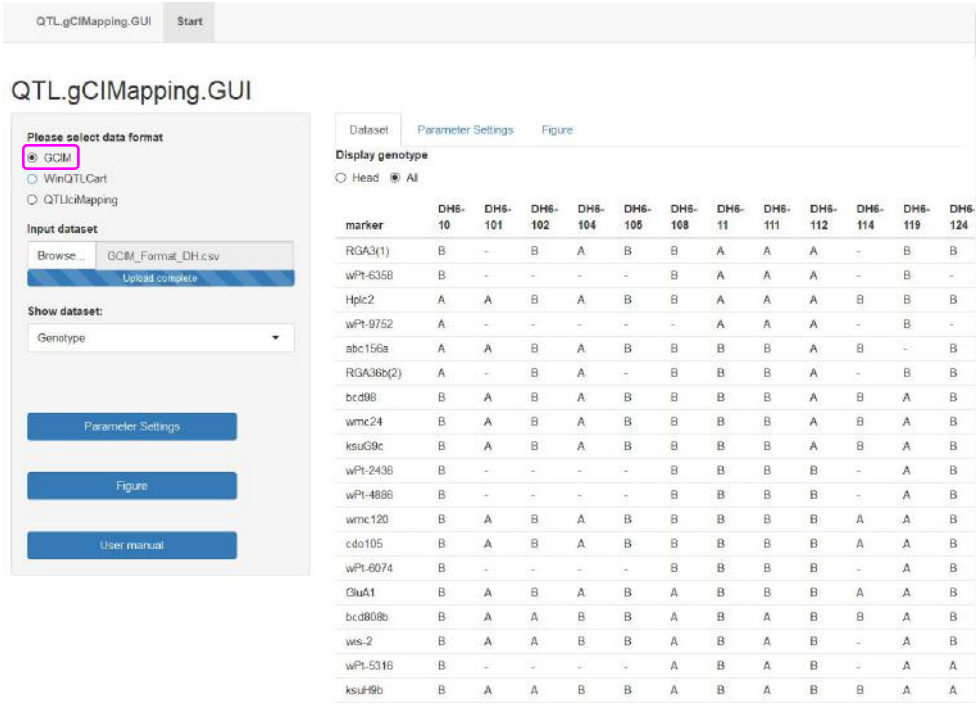
2. Wen Yang-Jun, Zhang Ya-Wen, Zhang Jin, Feng Jian-Ying, Jim M. Dunwell, Zhang Yuan-Ming*. An efficient multi-locus mixed model framework for the detection of small and linked QTLs in F2. Briefings in Bioinformatics 2019, 20(5): 1913-1924.

Figure 1. The Graphical User Interface of QTL.gCIMapping.GUI v2.1.1

3.2 Input dataset

Users must upload the dataset files with three kinds of formats (**Figs 2 to 4**). If users

select the QTLIciMapping format and there are the covariates, users should upload the covariate matrix (Fig 5).



marker	DH6-10	DH6-101	DH6-102	DH6-104	DH6-105	DH6-108	DH6-11	DH6-111	DH6-112	DH6-114	DH6-119	DH6-124
RGA3(1)	B	-	B	A	B	B	A	A	A	-	B	B
wPt-6358	B	-	-	-	-	B	A	A	A	-	B	-
Hplc2	A	A	B	A	B	B	A	A	A	B	B	B
wPt-9752	A	-	-	-	-	-	A	A	A	-	B	-
abc156a	A	A	B	A	B	B	B	B	A	B	-	B
RGA36b(2)	A	-	B	A	-	B	B	B	A	-	B	B
bcd98	B	A	B	A	B	B	B	B	A	B	A	B
wmc24	B	A	B	A	B	B	B	B	A	B	A	B
ksuG9c	B	A	B	A	B	B	B	B	A	B	A	B
wPt-2436	B	-	-	-	-	B	B	B	B	-	A	B
wPt-4886	B	-	-	-	-	B	B	B	B	-	A	B
wmc120	B	A	B	A	B	B	B	B	B	A	A	B
cdo105	B	A	B	A	B	B	B	B	B	A	A	B
wPt-6074	B	-	-	-	-	B	B	B	B	-	A	B
GluA1	B	A	B	A	B	A	B	B	B	A	A	B
bcd808b	B	A	A	B	B	A	B	A	B	B	A	B
wis-2	B	A	A	B	B	A	B	A	B	-	A	B
wPt-5316	B	-	-	-	-	A	B	A	B	-	A	A
ksuH9b	B	A	A	B	B	A	B	A	B	B	A	A

Fig 2. The GCIM dataset format

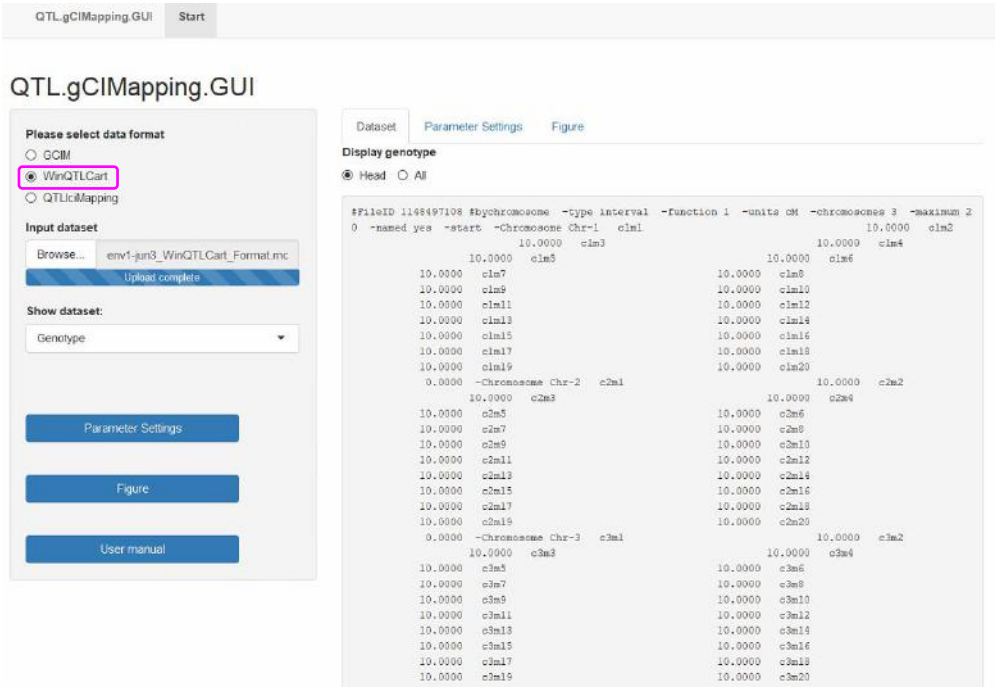
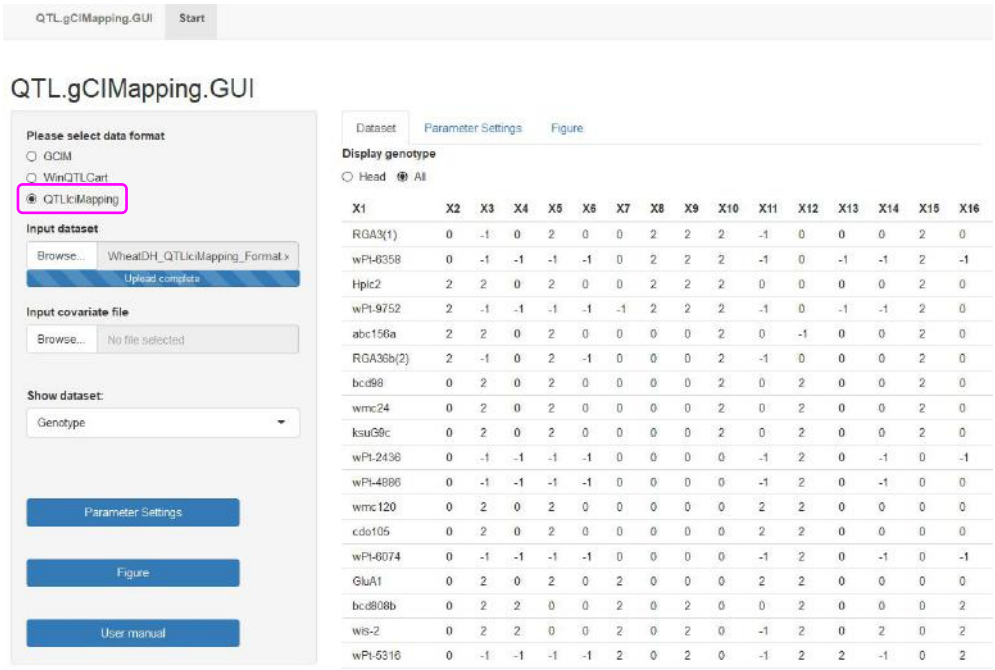
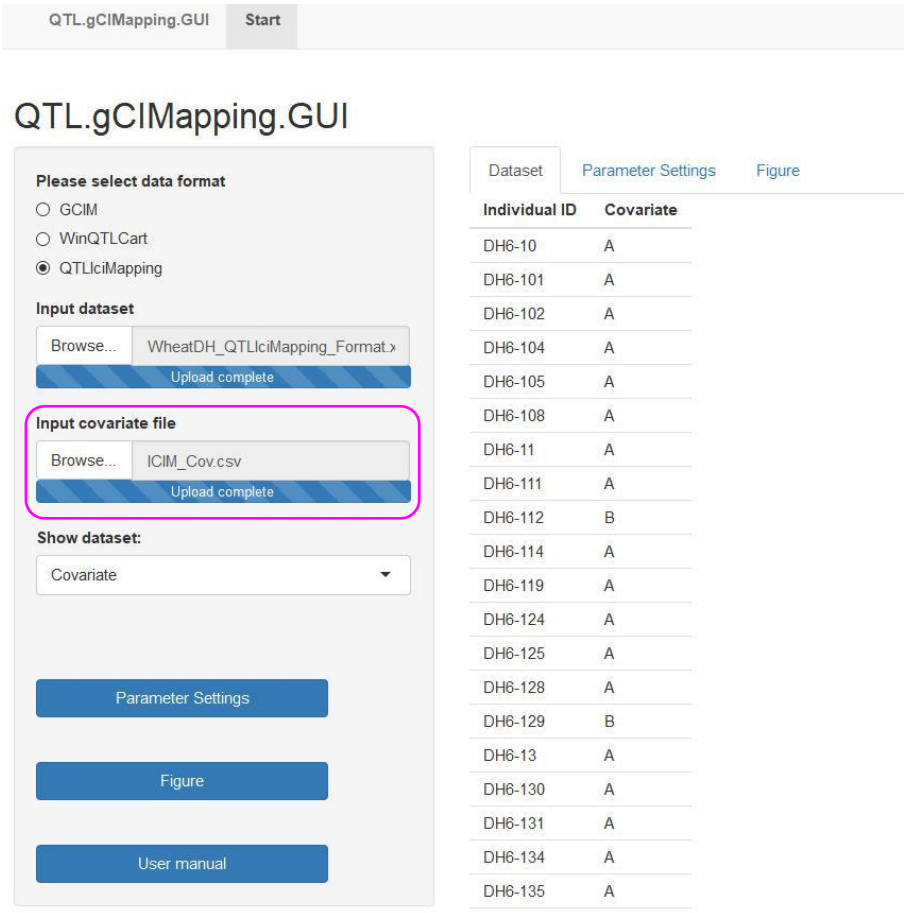


Fig 3. The WinQTLCart dataset format



X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16
RGA3(1)	0	-1	0	2	0	0	2	2	2	-1	0	0	0	2	0
wPt-6358	0	-1	-1	-1	-1	0	2	2	2	-1	0	-1	-1	2	-1
Hplc2	2	2	0	2	0	0	2	2	2	0	0	0	0	2	0
wPt-9752	2	-1	-1	-1	-1	-1	2	2	2	-1	0	-1	-1	2	0
abc156a	2	2	0	2	0	0	0	0	2	0	-1	0	0	2	0
RGA36b(2)	2	-1	0	2	-1	0	0	0	2	-1	0	0	0	2	0
bcd98	0	2	0	2	0	0	0	0	2	0	2	0	0	2	0
wmc24	0	2	0	2	0	0	0	0	2	0	2	0	0	2	0
ksuG9c	0	2	0	2	0	0	0	0	2	0	2	0	0	2	0
wPt-2436	0	-1	-1	-1	-1	0	0	0	0	-1	2	0	-1	0	-1
wPt-4886	0	-1	-1	-1	-1	0	0	0	0	-1	2	0	-1	0	0
wmc120	0	2	0	2	0	0	0	0	0	2	2	0	0	0	0
cdo105	0	2	0	2	0	0	0	0	0	2	2	0	0	0	0
wPt-6074	0	-1	-1	-1	-1	0	0	0	0	-1	2	0	-1	0	-1
GluA1	0	2	0	2	0	2	0	0	0	2	2	0	0	0	0
bcd808b	0	2	2	0	0	2	0	2	0	0	2	0	0	0	2
wis-2	0	2	2	0	0	2	0	2	0	-1	2	0	2	0	2
wPt-5316	0	-1	-1	-1	-1	2	0	2	0	-1	2	2	-1	0	2

Fig 4. The QTLIciMapping dataset format



Individual ID	Covariate
DH6-10	A
DH6-101	A
DH6-102	A
DH6-104	A
DH6-105	A
DH6-108	A
DH6-11	A
DH6-111	A
DH6-112	B
DH6-114	A
DH6-119	A
DH6-124	A
DH6-125	A
DH6-128	A
DH6-129	B
DH6-13	A
DH6-130	A
DH6-131	A
DH6-134	A
DH6-135	A

Fig 5. Covariate input in the QTLIciMapping dataset format

3.3 Parameter settings (Fig 6)

Select population: BC1 ($F_1 \times P_1$), BC2 ($F_1 \times P_2$), DH, RIL, and F_2.

Select model: Random or Fixed model for QTL effects.

Walk Speed for Genome-wide Scanning (cM): Set walk speed for genome-wide scanning (centi-Morgan, cM), for example, 1 cM.

Critical LOD score: Critical LOD scores for significant QTL, for example, 2.5 or 3.0.

Likelihood function: This parameter is only for F2 population, including restricted maximum likelihood (REML) and maximum likelihood (ML).

Random seeds: This parameter is only for F_2 population, in which the cross validation experiment is needed. Generally speaking, the random seed in the cross-validation experiment was set as 11001. If some known genes aren't identified by the seed, users may try to use some new random seeds. At this case, one better result may be obtained.

Completing CIM in one neighborhood: This parameter is only for F_2 population. In the first running, please set "FALSE". If the other software detects only one QTL in a neighborhood but the current software finds two linked QTLs (one with additive effect and another with dominant effect) in the neighborhood, please set "TRUE" and run again.

Traits analyzed: "**2**" indicates the genetic analyses for the **second** trait, "**2:4**" indicates the genetic analyses for the second **to** fourth traits, and "**2,4**" indicates the genetic analyses for the second **and** fourth traits.

Save path: The result will be written to the path in your computer.

Draw plot or not: This parameter setup includes FALSE and TRUE. "FALSE" indicates no figure output， and "TRUE" indicates the output of QTL mapping curve, for example, the LOD score [or $-\log_{10}(P\text{-value})$] curve against genome position.

Resolution of plot: Low or High: the low or high resolution for the figure file.

Plot format: Users can download the picture for different file formats: *.jpeg, *.png,

*.tiff and *.pdf.

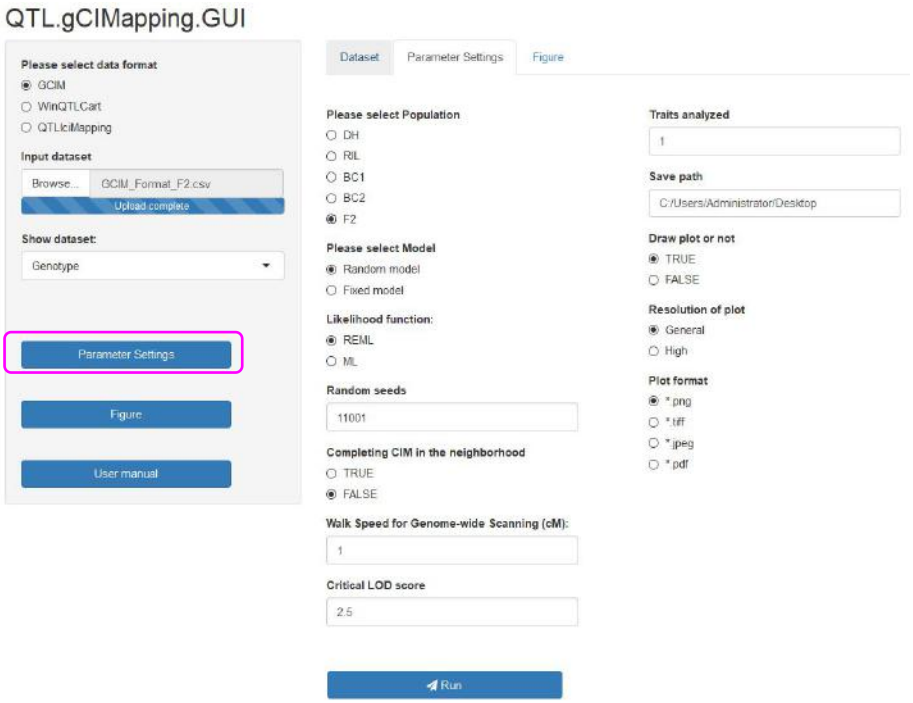


Fig 6. Parameter setting in the mapping of QTL for quantitative traits

3.4 Run the software

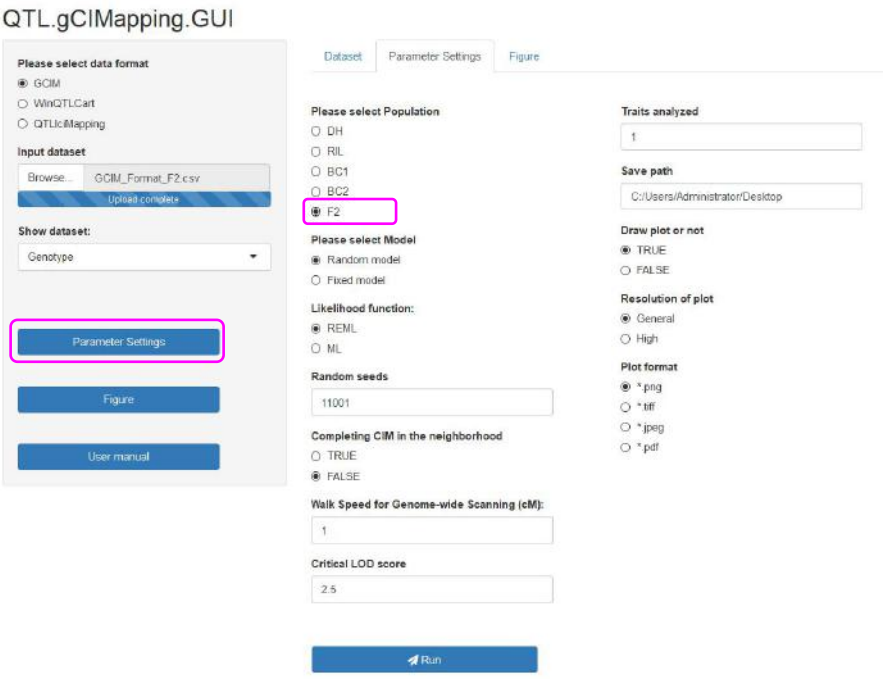


Fig 7. Run the software package QTL.gCIMapping.GUI v2.1.1

3.5 Re-draw the plot according to your own requirement

When users finish the running, users get the **resultforplot.csv** file. With this file information, users may redraw the curve figure {LOD score or $-\log_{10}(P\text{-value})$ }. With this Figure module, users may set all the figure parameters (**Fig 8**), including

Legend and tick marks: the size of the words in axis.

LOD line size: the size of the LOD line, the larger the coarser.

Size for $-\log_{10}$(P-value) curve: the size of $-\log_{10}$(P-value) curve, the larger the coarser.

Margin space: the space between the figure and the margin of the paper.

Critical LOD score: The critical LOD score for significant QTL.

Before saving this Figure, please set the related parameters: **width** and **height** [with the unit of pixel (px)], **word resolution** [with the unit of 1/72 inch, being pixels per inch (ppi)], and **figure resolution** [with the unit of pixels per inch (ppi)]. Users may set the colors for the LOD line color and $-\log_{10}$(P-value) curve, with a drop-down option. Use Download plot button to choose a path and to save the Figure, with four frequently used image formats: *.png, *.tiff , *.jpeg and *.pdf (**Fig 9**).

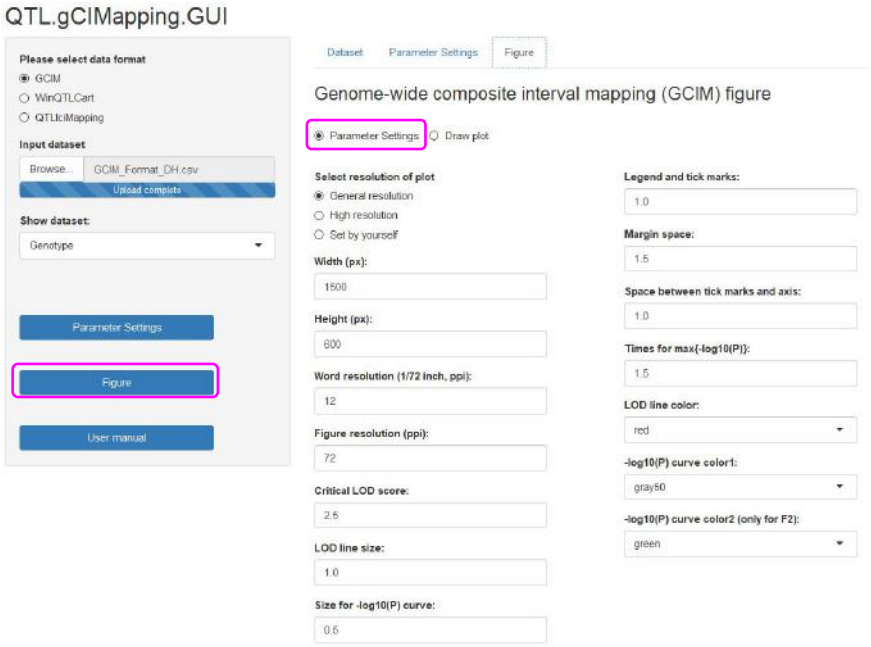


Figure 8. Parameter settings

Fig 9. How to draw the LOD score figure in QTL mapping

5 Result

Once the running of the software QTL.gCIMapping.GUI v2.1.1 ended, the "results" files would appear on the Directory, which was set up by users before running the software. The results for each trait include three files: "*_GCIM result.csv", "*_resultforplot.csv", and a GCIM plot.

In the *_GCIM result.csv file, there are ten columns for BC1, BC2, DH, and RIL populations, as shown below.

Trait: The trait name analyzed.

Chr: Chromosome, represented by an integer number.

Position (cM): The QTL position (cM) on the chromosome.

Additive Effect: Additive effect for significant QTL.

LOD: LOD score for significant QTL.

Left_Marker: Left flanking marker name for significant QTL.

Right_Marker: Right flanking marker name for significant QTL.

Var_Genet: Genetic variance for each significant QTL.

r^2 (%): Proportion of phenotypic variance explained by single QTL.

Var_Error: residual variance for the full model.

Var_Phen (total): Phenotypic variance in the analyzed population.

There are eleven columns for F_2 population, the Trait, Chr, Position (cM), Left_Marker, Right_Marker, Var_Genet, LOD, r^2 (%), Var_Error and Var_phen are same as those in the above-mentioned populations. In F_2 population, QTLs include additive (**Effect.a**) and dominant (**Effect.d**) effects.

In the "*_resultforplot.csv"file, there are six columns for BC1, BC2, DH, and RIL populations, as shown below.

Chr: Chromosome, represented by an integer number, for each potential QTL.

Position (cM): The QTL position (cM) on the chromosome, for each potential QTL.

-log$_{10}$(P-value): logarithm of P-value for each potential QTL.

Chr (Significant QTL): Chromosome, represented by an integer number, for each significant QTL.

Position (cM) (Significant QTL): The QTL position (cM) of each significant QTL on the chromosome.

LOD (Significant QTL): logarithm of odds for each significant QTL.

There are seven columns for F_2 populations, the Chr, Position (cM), Chr (Significant QTL), Position (cM) (Significant QTL), LOD (Significant QTL) are same as those in the above populations. In F_2 population, QTLs include P-value of additive effects (**'-log10(P-value)(a)'**) and dominant effects (**'-log10(P-value)(d)'**).

In the GCIM plot, the -log$_{10}$(P) values are indicated by curves, if F_2 population is selected, there will be two curves in the GCIM plot, one is for additive effect and another is for dominant effect. All the significant QTLs identified are indicated by the vertical lines.

Appendix. The R codes for drawing Figure 3 in the third reference

```
######The input files are the result files from the GCIM, ICIM and CIM methods

######There are six columns in the GCIM result file, including chromosome, ######marker position (cM),
P-value, the chromosome of the QTLs identified,
######the position of the QTLs detected, and the LOD score of the QTLs mapped.

######There are three columns in the CIM and ICIM result file, including
######chromosome, position (cM) and LOD score for each putative QTLs.

rm(list=ls())
library("data.table")
setwd("E:/QTL_plot/")
gcimFunc <- function(mxmp,galaxyy1,res11,chr_name,method)
{
  chr_pos <- mxmp[,1:2]
  chr_num <- length(chr_name)
  chr <- matrix(0,chr_num,1)
  pos <- matrix(0,chr_num,1)
  for(i in 1:chr_num)
  {
    temp <- numeric()
    temp <- length(which(chr_pos[,1]==i))
    if(i==1)
    {
      pos[i] <- temp
      chr[i] <- chr_pos[pos[i],2]
    }else{
      pos[i] <- pos[i-1] + temp
      chr[i] <- chr_pos[pos[i],2]
    }
  }

  pos_acc <- matrix(0,chr_num,1)
  for(i in 1:chr_num)
  {
    if(i==1){
      pos_acc[i] <- chr[i]
    }else{
      pos_acc[i] <- pos_acc[i-1] + chr[i]
    }
  }
```

```
firFil <- res11[,1:2]
newposadd <- as.matrix(firFil[,2])
for(i in 1:chr_num)
{
   temp1 <- numeric()
   temp1 <- which(firFil[,1]==i)
   if(i>1)
   {
      newposadd[temp1] <- newposadd[temp1]+pos_acc[i-1]
   }
}

if(method=="GCIM"){

   if(is.null(galaxyy1)==FALSE){
      if(is.null(dim(galaxyy1))==TRUE){
         galaxyy1<-matrix(galaxyy1,1,3)
      }
      newres_pos <- galaxyy1[,2]
      res_sumpos <- pos_acc[galaxyy1[which(galaxyy1[,1]>1),1]-1] + galaxyy1[which(galaxyy1[,1]>1),2]
      newres_pos[which(galaxyy1[,1]>1)] <- res_sumpos
      pospic<-c(newres_pos)
      lodpic<-c(galaxyy1[,3])
      resdf <- data.frame(pospic,lodpic)
   }

   resp<-as.matrix(res11[,3])
   pmin<-min(resp[resp!=0])
   locsub<-which(resp==0)
   if(length(locsub)!=0){
      subvalue<-10^(1.1*log10(pmin))
      res11[locsub,3]<-subvalue
   }else{
      res11<-res11
   }
   negloP <- -log10(as.matrix(res11[,3]))
   fanhui<-list(newposadd,negloP,pospic,lodpic,pos_acc)

}else{

   lodpic<-res11[,3]
   fanhui<-list(newposadd,lodpic,pos_acc)
}
```

```
  return(fanhui)
}
##################    data processing    ############################
##################            GCIM            ############################

data_plot_gcim<-as.matrix(fread("GCIM_draw.csv"))
res11_GCIM<-data_plot_gcim
mxmp_GCIM<-data_plot_gcim[,1:2]
chr_name_GCIM<-unique(data_plot_gcim[,1])
galaxyy11<-data_plot_gcim[,4:6]
galaxyy1_GCIM<-matrix(galaxyy11[1:which(is.na(galaxyy11)==TRUE,arr.ind = TRUE)[1,1]-1,],,3)
method_GCIM="GCIM"
Fun_result_GCIM<-gcimFunc(mxmp_GCIM,galaxyy1_GCIM,res11_GCIM,chr_name_GCIM,method_GCIM)
newposadd_GCIM<-Fun_result_GCIM[[1]]##### Genome position
negloP_GCIM<-Fun_result_GCIM[[2]]######## -log10(P-value) curve
pospic_GCIM<-Fun_result_GCIM[[3]]########position of significant QTL
lodpic_GCIM<-Fun_result_GCIM[[4]]######## LOD score of significant QTL
pos_acc_GCIM<-Fun_result_GCIM[[5]]####### Chromosomal boundary

######### ICIM ##########
data_plot_icim<-as.matrix(fread("ICIM_draw.csv"))
res11_ICIM<-data_plot_icim
mxmp_ICIM<-data_plot_icim[,1:2]
chr_name_ICIM<-unique(data_plot_icim[,1])
method_ICIM="ICIM"
Fun_result_ICIM<-gcimFunc(mxmp_ICIM,galaxyy1=NULL,res11_ICIM,chr_name_ICIM,method_ICIM)
newposadd_ICIM<-Fun_result_ICIM[[1]]##### Genome position
lodpic_ICIM<-Fun_result_ICIM[[2]]######## LOD score curve
pos_acc_ICIM<-Fun_result_ICIM[[3]]####### Chromosomal boundary

########### CIM ##############
data_plot_cim<-as.matrix(fread("CIM_draw.csv"))
res11_CIM<-data_plot_cim
mxmp_CIM<-data_plot_cim[,1:2]
chr_name_CIM<-unique(data_plot_cim[,1])
method_CIM="CIM"
Fun_result_CIM<-gcimFunc(mxmp_CIM,galaxyy1=NULL,res11_CIM,chr_name_CIM,method_CIM)
newposadd_CIM<-Fun_result_CIM[[1]]##Genome position
lodpic_CIM<-Fun_result_CIM[[2]]##LOD score curve
pos_acc_CIM<-Fun_result_CIM[[3]]##Chromosomal boundary
LODmax<-max(lodpic_GCIM,lodpic_ICIM,lodpic_CIM)##Max value of left vertical axis

############# Parameter settings in plot ######################
legend_size<-1.0##Size of vertical label
```

```
mainline_size<-1.5##Size of LOD score in GCIM
backline_size<-1.5##Size of curves
axis_space<-1.0##Distance between axis and graph
logPCoff<-1.8##Times for max{-log10(P)}
color1<-"blue"##LOD score
color2<-"gray50"##-log10(P-value) curve
lodthred<-2.5##The critical LOD score
a<-1;b<-5;c<-0;d<-5##Distance between graph and border(bottom,left,top,right)
ztcex=1.3##size of text

################ To draw plot                ##########################
pdf("Plot_3.pdf",width=11)
layout(matrix(1:3,ncol = 1),heights=c(1.15,1,1.25))

############### To draw plot for GCIM    ##########################
par(mar=c(a,b,2,d),mgp=c(3*axis_space,axis_space,0))
plot(newposadd_GCIM,negloP_GCIM,type="l",col=color2,xaxt="n",yaxt="n",
     xlab="",ylab="",lwd=backline_size,xlim=c(0,max(newposadd_GCIM)),
     ylim=c(0,logPCoff*max(negloP_GCIM)),bty="u")##Draw -log10(P-value) curve
axis(side=4,family="serif",cex.axis=1.5)
abline(v=pos_acc_GCIM,lty=2,col="gray")##Draw chromosomal boundary
par(new=TRUE)
plot(pospic_GCIM,lodpic_GCIM,type="h",col=color1,xaxt="n",yaxt="n",xlab="",ylab="",
     cex.axis=legend_size,cex.lab=1.5,lwd=mainline_size,xlim=c(0,max(newposadd_GCIM)),
     ylim=c(0,LODmax),bty="l",family="serif")##LOD score of significant QTL
box(bty="o",lwd=1.5)
axis(side=2,family="serif",cex.axis=1.5)
mtext("LOD score",side=2,line=3*axis_space,cex=legend_size,col=color1,family="serif")
abline(h=lodthred,lty=5,col="azure4")##critical LOD score
mtext(expression('-log'[10]*'(P-value)'),side=4,
      line=3*axis_space,cex=legend_size,family="serif",col=color2)

######    Mark gene or QTL    ########
text(34,21,"kgw1a",cex = ztcex,family="serif")
text(77,6.5,"RDD1",cex = ztcex,family="serif",font=3)
text(145,10,"gw-1",cex = ztcex,family="serif")
text(350,12,"KRP1",cex = ztcex,family="serif",font=3)
text(470,26,"GS3",cex = ztcex,family="serif",font=3)
text(530,12,"kgw3b",cex = ztcex,family="serif")
text(720,36,"GW5",cex = ztcex,family="serif",font=3)
text(767,6.3,"OsSec18",cex=ztcex,family="serif",font=3)
text(810,9.3,"OsACS6",cex = ztcex,family="serif",font=3)
text(855,13,"PFP",cex = ztcex,family="serif",font=3)
text(886,13,expression(italic(beta)),cex=ztcex)
```

```
text(906,7.8,"tgw6",cex = ztcex,family="serif")
text(970,7,"gw7.1",cex = ztcex,family="serif")
text(1290,14.3,"OsSPL18",cex = ztcex,family="serif",font = 3)
text(1460,8.5,"gw11",cex = ztcex,family="serif")
text(1300,36.5,"Genome-wide Composite Interval Mapping (GCIM)",cex = ztcex+0.5,family="serif")

###### To draw plot for ICIM        #########
par(mar=c(a,b,c,d),mgp=c(3*axis_space,axis_space,0))
plot(newposadd_ICIM,lodpic_ICIM,type="l",col=color1,xaxt="n",yaxt="n",xlab="",
        ylab="",lwd=backline_size,
        xlim=c(0,max(newposadd_ICIM)),ylim=c(0,max(lodpic_ICIM)),
        cex.axis=legend_size,cex.lab=1.5,bty="l",family="serif")##Draw LOD score curve
box(bty="u",lwd=1.5)
axis(side=2,family="serif",cex.axis=1.5)
mtext("LOD score",side=2,line=3*axis_space,cex=legend_size,col=color1,family="serif")
abline(h=lodthred,lty=5,col="azure4")
abline(v=pos_acc_ICIM,lty=2,col="gray")

###### Mark gene or QTL #####
text(34,25,"kgw1a",cex = ztcex,family="serif")
text(77,10.5,"RDD1",cex = ztcex,family="serif",font=3)
text(145,10,"gw-1",cex = ztcex,family="serif")
text(350,12,"KRP1",cex = ztcex,family="serif",font=3)
text(680,37,"GW5",cex = ztcex,family="serif",font=3)
text(810,9.3,"OsACS6",cex = ztcex,family="serif",font=3)
text(960,8.5,"gw7.1",cex = ztcex,family="serif")
text(1010,5.3,"Kw7-2",cex = ztcex,family="serif")
text(1460,8.9,"gw11",cex = ztcex,family="serif")
text(1350,38,"Inclusive Composite Interval Mapping (ICIM)",cex = ztcex+0.5,family="serif")

###### Draw plot for CIM #########
par(mar=c(5,b,c,d),mgp=c(3*axis_space,axis_space,0))
plot(newposadd_CIM,lodpic_CIM,type="l",col=color1,yaxt="n",
        ylab="",lwd=backline_size,
        xlim=c(0,max(newposadd_CIM)),ylim=c(0,LODmax),
        cex.axis=1.5,cex.lab=2.0,bty="l",family="serif",
        xlab="Genome position (cM)")##The LOD score curve
box(bty="u",lwd=1.5)
axis(side=2,family="serif",cex.axis=1.5)
mtext("LOD score",side=2,line=3*axis_space,cex=legend_size,col=color1,family="serif")
abline(h=lodthred,lty=5,col="azure4")
abline(v=pos_acc_CIM,lty=2,col="gray")

###### Mark gene or QTL #####
```

```
text(34,11,"kgw1a",cex = ztcex,family="serif")
text(79,6,"RDD1",cex = ztcex,family="serif",font=3)
text(145,8,"gw-1",cex = ztcex,family="serif")
text(470,20,"GS3",cex = ztcex,family="serif",font=3)
text(720,23,"GW5",cex = ztcex,family="serif",font=3)
text(950,4.5,"gw7.1",cex = ztcex,family="serif")
text(1030,4.5,"Kw7-2",cex = ztcex,family="serif")
text(1290,9.3,"OsSPL18",cex = ztcex,family="serif",font = 3)
text(1460,8.5,"gw11",cex = ztcex,family="serif")
text(1420,39,"Composite Interval Mapping (CIM)",cex = ztcex+0.5,family="serif")

dev.off()
```