

User Manual for

QTL.gCIMapping.GUI

QTL genome-wide Composite Interval Mapping GUI

(version 2.0+)

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Last updated on April 17, 2020

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>).

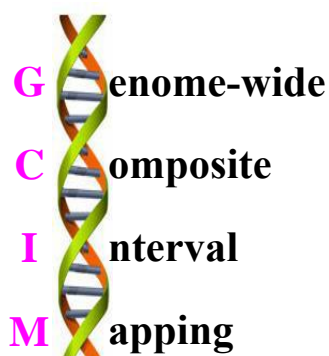
Download website:

<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 F₂. *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*, in revision.

Quantitative Trait Loci



Funding

The works were supported by the National Natural Science Foundation of China (31571268, 31871242, 31701071 and U1602261), Huazhong Agricultural University Scientific & Technological Self-innovation Foundation (Program No. 2014RC020), and State Key Laboratory of Cotton Biology Open Fund (CB2017B01 and CB2019B01).

INTRODUCTION

1.1 The function of the GCIM software

QTL.gCIMapping.GUI v2.0+ (**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 (IF₂). QTL.gCIMapping.GUI v2.0+ works well on the R environment on Windows, Linux (desktop) and MacOS.

1.2 Getting started

The software package QTL.gCIMapping.GUI v2.0+ 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 (soyzzhang@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 v2.0+.

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

"cmprsk", "corpcor", "data.table", "digest", "doParallel", "Epi", "etm", "fdrtool", "foreach", "GeneNet", "glmnet", "htmltools", "httpuv", "iterators", "jsonlite", "later", "longitudinal", "magrittr", "MASS", "mime", "numDeriv", "openxlsx", "parcor", "plyr", "ppals", "promises", "QTL.gCIMapping", "qtl", "R6", "Rcpp", "shiny", "sourcetools", "stringi", "stringr", "testthat", "utf8", "xtable", "zip", "zoo",

from CRAN, github (<https://github.com/>), or google search.

On the R environment, then, users select all the 39 packages and install them offline.

1.2.2.2 Install QTL.gCIMapping.GUI v2.0+

On R GUI environment, users first select ["Packages"—"Install package\(s\) from local files..."](#), then find the software package [QTL.gCIMapping.GUI v2.0+](#) on user's desktop computer or mobile device, and launch [QTL.gCIMapping.GUI v2.0+](#).

1.2.3 Run QTL.gCIMapping.GUI v2.0+

Once the software package QTL.gCIMapping.GUI v2.0+ 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 v2.0+, 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. 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 v2.0+

QTL.gCIMapping.GUI (QTL genome-wide Composite Interval Mapping with Graphical User Interface)

Coding criteria			Dataset example					
Genotype	Code	Meaning	marker	chr	pos	DH6.10	DH6.101	DH6.102
AA	A	Homozygous genotype (P1)	RGA3(1)	1	0	B	-	B
Aa	H	Heterozygous genotype (F1)	wPt-6358	1	3.034	B	-	-
aa	B	Homozygous genotype (P2)	Hplc2	1	8.8291	A	A	B
AA+Aa(Not aa)	D	Dominance to P1
Aa+aa(Not AA)	C	Dominance to P2	gwm437	21	162.5218	A	B	-
Missing	-	Missing or unclear genotype	gwm121	21	180.2878	A	B	-
			wmc157	21	197.9196	A	B	A
			trait1	T19	75.33	105	96.33	
			trait2	T191	74	105.68	97.16	
			Covar	CovarName	A	B	B	

Reference

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.
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, in revision.

Figure 1. The Graphical User Interface of QTL.gCIMapping.GUI v2.0+

3.2 Input dataset

Users must upload the dataset files with three kinds of formats (Figs 2 to 4). If users select the QTLgCIMapping format and there are the covariates, users should upload the

covariate matrix (Fig 5).

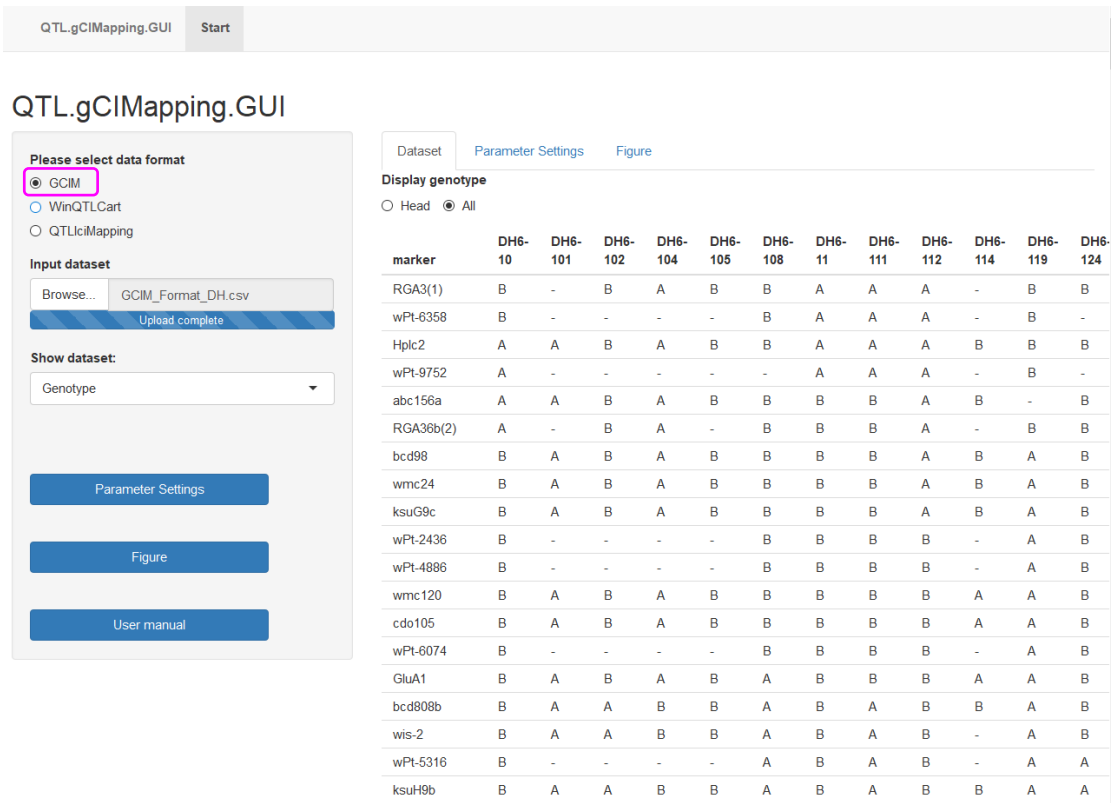


Fig 2. The GCIM dataset format

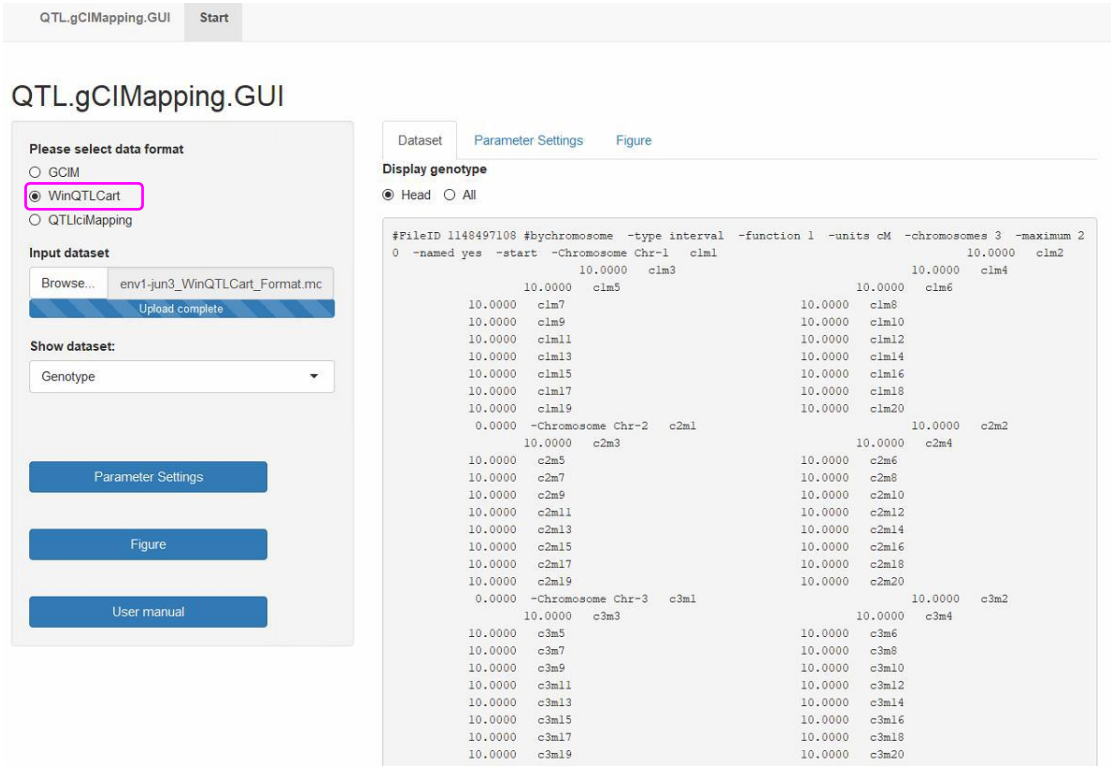


Fig 3. The WinQTLCart dataset format

QTL.gCIMapping.GUI

Start

QTL.gCIMapping.GUI

Please select data format

☐ GCIM
 ☐ WinQTLCart
 ☒ QTLiCiMapping

Input dataset

Browse...

WheatDH_QTLiCiMapping_Format.x

Upload complete

Input covariate file

Browse...

No file selected

Show dataset:

Genotype

Parameter Settings

Figure

User manual

Dataset

Parameter Settings

Figure

Display genotype

☐ Head
 ☒ All

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

QTL.gCIMapping.GUI

Start

QTL.gCIMapping.GUI

Please select data format

☐ GCIM
 ☐ WinQTLCart
 ☒ QTLiCiMapping

Input dataset

Browse...

WheatDH_QTLiCiMapping_Format.x

Upload complete

Input covariate file

Browse...

ICIM_Cov.csv

Upload complete

Show dataset:

Covariate

Parameter Settings

Figure

User manual

Dataset

Parameter Settings

Figure

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 F_2 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: "2:2" or "2" indicates the analyses from the second trait, "2:4" indicates the analyses from the second to fourth traits, and "2,4" indicates the analyses of the second trait and the fourth trait.

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.

QTL.gCIMapping.GUI

Please select data format

☒ GCIM

☐ WinQTLCart

☐ QTL IciMapping

Input dataset

Browse... GCIM_Format_F2.csv

Upload complete

Show dataset:

Genotype

Parameter Settings

Figure

User manual

Please select Population

☐ DH

☐ RIL

☐ BC1

☐ BC2

☒ F2

Please select Model

☒ Random model

☐ Fixed model

Likelihood function:

☒ REML

☐ ML

Random seeds

11001

Completing CIM in the neighborhood

☐ TRUE

☒ FALSE

Walk Speed for Genome-wide Scanning (cM):

1

Critical LOD score

2.5

Run

Traits analyzed

1

Save path

C:/Users/Administrator/Desktop

Draw plot or not

☒ TRUE

☐ FALSE

Resolution of plot

☒ General

☐ High

Plot format

☒ *.png

☐ *.tiff

☐ *.jpeg

☐ *.pdf

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

3.4 Run the software

QTL.gCIMapping.GUI

Please select data format

☒ GCIM

☐ WinQTLCart

☐ QTL IciMapping

Input dataset

Browse... GCIM_Format_F2.csv

Upload complete

Show dataset:

Genotype

Parameter Settings

Figure

User manual

Please select Population

☐ DH

☐ RIL

☐ BC1

☐ BC2

☒ F2

Please select Model

☒ Random model

☐ Fixed model

Likelihood function:

☒ REML

☐ ML

Random seeds

11001

Completing CIM in the neighborhood

☐ TRUE

☒ FALSE

Walk Speed for Genome-wide Scanning (cM):

1

Critical LOD score

2.5

Run

Traits analyzed

1

Save path

C:/Users/Administrator/Desktop

Draw plot or not

☒ TRUE

☐ FALSE

Resolution of plot

☒ General

☐ High

Plot format

☒ *.png

☐ *.tiff

☐ *.jpeg

☐ *.pdf

Fig 7. Run the software package QTL.gCIMapping.GUI v2.0+

3.5 Re-draw the plot according to your own requirement

When users finish the running, users get the resultforplot.xlsx 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 $-\log_{10}(P\text{-value})$ curve: the size of $-\log_{10}(P\text{-value})$ 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 $-\log_{10}(P\text{-value})$ 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).

QTL.gCIMapping.GUI

Please select data format

☒ GCIM

☐ WinQTLCart

☐ QTLciMapping

Input dataset

Browse... GCIM_Format_DH.csv

Upload complete

Show dataset:

Genotype

Parameter Settings

Figure

User manual

Dataset Parameter Settings Figure

Genome-wide composite interval mapping (GCIM) figure

☒ Parameter Settings ☐ Draw plot

Select resolution of plot

☒ General resolution

☐ High resolution

☐ Set by yourself

Width (px):

1500

Height (px):

600

Word resolution (1/72 inch, ppi):

12

Figure resolution (ppi):

72

Critical LOD score:

2.5

LOD line size:

1.0

Size for $-\log_{10}(P)$ curve:

0.5

Legend and tick marks:

1.0

Margin space:

1.5

Space between tick marks and axis:

1.0

Times for max($-\log_{10}(P)$):

1.5

LOD line color:

red

$-\log_{10}(P)$ curve color1:

gray50

$-\log_{10}(P)$ curve color2 (only for F2):

green

Figure 8. Parameter settings

QTL.gCIMapping.GUI



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

4 Result

For BC1, BC2, DH and RIL populations, the **Results** file has ten columns, 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.

r² (%): 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.

For F₂ population, the **Results** file has eleven columns. Trait, Chr, Position (cM), Left_Marker, Right_Marker, Var_Genet, LOD, r² (%), Var_Error and Var_phen are same as those in the above populations. In F₂ population, QTLs include additive (**Effect.a**) and dominant (**Effect.d**) effects.

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])
galaxyy1<-data_plot_gcim[,4:6]
galaxyy1_GCIM<-matrix(galaxyy1[1:which(is.na(galaxyy1))==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_G
CIM)
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_ICI
M)
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="",yla
b="",
cex.axis=legend_size,cex.lab=1.5,lwd=mainline_size,xlim=c(0,max(newposadd_GCI
M)),
      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()

```