# Package 'GALLO'

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**Title** Genomic Annotation in Livestock for Positional Candidate LOci

**Version** 1.5

**Description** The accurate annotation of genes and Quantitative Trait Loci (QTLs) located within candidate markers and/or regions (haplotypes, windows, CNVs, etc) is a crucial step the most common genomic analyses performed in livestock, such as Genome-Wide Association Studies or transcriptomics. The Genomic Annotation in Livestock for positional candidate LOci (GALLO) is an R package designed to provide an intuitive and straightforward environment to annotate positional candidate genes and QTLs from high-throughput genetic studies in livestock. Moreover, GALLO allows the graphical visualization of gene and QTL annotation results, data comparison among different grouping factors (e.g., methods, breeds, tissues, statistical models, studies, etc.), and QTL enrichment in different livestock species including cattle, pigs, sheep, and chicken, among others.

**URL** <[https://github.com/pablobio/GALLO](https://github.com/pablobio/GALLO)>

**Depends** R (>= 4.0.0)

**biocViews** Software

**Imports** circlize, data.table, doParallel, dplyr, ggplot2, graphics, grDevices, foreach, lattice , parallel, RColorBrewer, rtracklayer, stats, stringr, unbalhaar, utils, DT, webshot, igraph, visNetwork, CompQuadForm, Matrix

**License** GPL-3

**Encoding** UTF-8

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**RoxygenNote** 7.2.1

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**VignetteBuilder** knitr

**NeedsCompilation** no

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find_genes_qtls_around_markers

*Search genes and QTLs around candidate regions*

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## Description

Takes a list of candidate markers and or regions (haplotypes, CNVs, windows, etc.) and search for
genes or QTLs in a determined interval

## Usage

```
find_genes_qtls_around_markers(
  db_file,
  marker_file,
  method = c("gene", "qtl"),
  marker = c("snp", "haplotype"),
  interval = 0,
  nThreads = NULL,
  verbose = TRUE
)
```

**Arguments**

| | |
|---|---|
| db_file | The data frame obtained using the import_gff_gtf() function |
| marker_file | The file with the SNP or haplotype positions. Detail: For SNP files, the columns "CHR" and "BP" with the chromosome and base pair position, respectively, are mandatory. For the haplotype, the following columns are mandatory: "CHR", "BP1" and "BP2" |
| method | "gene" or "qtl" |
| marker | "snp" or "haplotype" |
| interval | The interval in base pair which can be included upstream and downstream from the markers or haplotype coordinates. |
| nThreads | Number of threads to be used |
| verbose | Logical value defining if messages should of not be printed during the analysis (default=TRUE) |

**Value**

A dataframe with the genes or QTLs mapped within the specified intervals

**Examples**

```
data(QTLmarkers)
data(gffQTLs)
out.qtls<-find_genes_qtls_around_markers(db_file=gffQTLs, marker_file=QTLmarkers,
method = "qtl", marker = "snp",
interval = 500000, nThreads = 1)
```

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| gene_pval | *Estimate a gene-level p-value using Weighted Z-score approach and Meta-analysis with LD correlation coefficients approach* |
|---|---|

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**Description**

Estimate a gene-level p-value using Weighted Z-score approach and Meta-analysis with LD correlation coefficients approach

**Usage**

```
gene_pval(data, db_file, marker_ld, interval, p)
```

**Arguments**

| | |
|---|---|
| data | A data frame with the results of the association test performed for each marker |
| db_file | A data frame obtained from the import_gff_gtf containing the gtf information |
| marker_ld | A data frame containing the pairwise linkage disequilibrium between markers in a chromosome |

| interval | The interval (in base pairs) used to annotated markers downstream and upstream from the genes coordinates |
| --- | --- |
| p | The name of the column containing the P-values for each marker |

## Details

Requires a table with p-values from a association test, a gtf file file the gene coordinates in the same assembly used to map the variants used in the association study, and a data frame with pairwise linkage disequilibrium (LD) values between markers. This analysis must be performed for each chromosome individually. The data frame with the results of the association study must have three mandatory columns names as CHR, BP and SNP containing the chromosome, base pair position and marker name, respectively. The gtf file must be imported by the import_gff_gtf() function from GALLO or can be customized by the user, since it has the same columns names. The LD table must contain three mandatory columns, SNP_A, SNP_B and R. where, the first two columns must contain the marker names and the third column, the LD value between these markers. This dtaa frame can be obtained using PLINK or any other software which computes pairwise LD between markers in the same chromosome. In the absence of LD values between any two SNPs in the data frame, a LD equal zero is assumed

## Value

A data frame with the gene level p-values obtained using the Weighted Z-score approach (P_WZ_ld) and Meta-analysis with LD correlation coefficients approach (P_meta_LD)

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| import_gff_gtf | *Import .gtf and .gff files to be used during gene and QTL annotation, respectively* |
| --- | --- |

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## Description

Takes a .gft or .gff file and import into a dataframe

## Usage

```
import_gff_gtf(db_file, file_type)
```

## Arguments

| db_file | File with the gene mapping or QTL information. For gene mapping, a .gtf file from Ensembl database must be used. For the QTL search, a .gff file from Animal QTLdb must be used. Both files must use the same reference annotation used in the original study |
| --- | --- |
| file_type | "gtf" or "gff" |

## Value

A dataframe with the gtf or gtf content

## Examples

```
gffpath <- system.file("extdata", "example.gff", package="GALLO")

qtl.inp <- import_gff_gtf(db_file=gffpath,file_type="gff")
```

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| NetCen | *Compute the centrality metrics for the nodes composing the network generated by the NetVis function* |
|---|---|

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## Description

Compute the centrality metrics for the nodes composing the network generated by the NetVis function

## Usage

```
NetCen(data, g1, g2)
```

## Arguments

| | |
|---|---|
| data | A data frame containing the relationship between the two groups to be represented in the network |
| g1 | Name of the column containing the labels of the first group that will be used to create the network |
| g2 | Name of the column containing the labels of the second group that will be used to create the network |

## Details

This function returns the following centrality metrics for each node that composed the network: Degree (The number of edges incident to the node), Betweenness (The fraction of shortest paths between pairs of nodes that pass through the node), Closeness (The inverse of the sum of the shortest path distances from the node to all other nodes), and Eigenvector Centrality (The centrality measure based on the eigenvector of the adjacency matrix).

## Value

A data frame with the centrality metrics for each node in the network.

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| NetVis | *Create a dynamic network representing the relationship between two groups of variables* |
|---|---|

---

### Description

Create a dynamic network representing the relationship between two groups of variables

### Usage

```
NetVis(
  data,
  g1,
  g2,
  col1 = "aquamarine",
  col2 = "red",
  edge_col = "gray",
  remove_label = NULL,
  node_size = c(15, 40),
  font_size = 45,
  edge_width = 1
)
```

### Arguments

| | |
|---|---|
| data | A data frame containing the relationship between the two groups to be represented in the network |
| g1 | Name of the column containing the labels of the first group that will be used to create the network |
| g2 | Name of the column containing the labels of the second group that will be used to create the network |
| col1 | Color of the nodes that will represent the first group represented in the network. The default value is aquamarine |
| col2 | Color of the nodes that will represent the second group represented in the network The default value is red |
| edge_col | Color of the edges that will connect the nodes in the network. The default value is gray |
| remove_label | If is required to omit the labels for some of the groups, this argument receives the column name informed the g1 or g2 arguments. The default value is NULL |
| node_size | A vector with the node sizes to represent g1 and g2. The defaul values are 15 and 40, respectively |
| font_size | The size of the font of the labels of each node (The default value is 45) |
| edge_width | The width of the edges connecting the nodes in the network |

## Details

This function returns a dynamic network, using visNetwork, representing the connection between two groups. For example, the output from the find_genes_qtls_around_markers() function can be used here to represent the connections between markers and QTLs. Another option is to combine the data frames with both gene and QTL annotation around markers to reprsent the connections between genes and QTLs.

## Value

A dynamic network representing the connection between two groups.

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| Nmarkers_SimpleM | *Estimate the number of effective markers in a chromosome based on an adapted version of the simpleM methodology* |
|---|---|

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## Description

Estimate the number of effective markers in a chromosome based on an adapted version of the simpleM methodology

## Usage

```
Nmarkers_SimpleM(ld.file, PCA_cutoff = 0.995)
```

## Arguments

| | |
|---|---|
| ld.file | A data frame with the pairwise linkage disequilibrium (LD) values for a chromosome. The column names SNP_A, SNP_B, and R are mandatory, where the SNP_A and SNP_B contained the markers names and the R column the LD values between the two markers. |
| PCA_cutoff | A cutoff for the total of the variance explained by the markers. |

## Details

This function estimate the effective number of markers in a chromosome using adapted version of the simpleM methodology described in Gao et al. (2008). The function use as input a data frame composed by three mandatory columns (SNP_A, SNP_B, and R). This data frame can be obtained using PLINK or any other software to compute LD between markers. Additionally, a threshold for percentage of the sum of the variances explained by the markers must be provided. The number of effective markers identified by this approach can be used in multiple testing corrections, such as Bonferroni.

## Value

The effective number of markers identified by the SimpleM approach

## References

Gao et al. (2008) Genet Epidemiol, Volume 32, Issue 4, Pages 361-369. (doi:10.1002/gepi.20310)

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| Nseg_chr | *Estimate the number of independent segments in a chromosome based on the effective population size* |
|---|---|

---

## Description

Estimate the number of independent segments in a chromosome based on the effective population size

## Usage

```
Nseg_chr(chr.table, chr_length, Ne)
```

## Arguments

chr.table
: A table containing the chromosomes and the chromosomal length (in centiMorgans).

chr_length
: The name of the column where the length of the chromosomes are informed.

Ne
: The effective population size.

## Details

This function uses a adapted version of the formula proposed by Goddard et al. (2011) to estimate the independent number of segments in a chromosome based on the effective population size.

## Value

A data frame with the effevtive number of segments in each chromosome.

## References

Goddard et al. (2011) Journal of animal breeding and genetics, Volume 128, Issue 6, Pages 409-421. (doi:10.1111/j.14390388.2011.00964.x)

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overlapping_among_groups
*Overlapping between grouping factors*

---

### Description

Takes a dataframe with a column of genes, QTLs (or other data) and a grouping column and create some matrices with the overlapping information

### Usage

```
overlapping_among_groups(file, x, y)
```

### Arguments

| | |
|---|---|
| file | A dataframe with the data and grouping factor |
| x | The grouping factor to be compared |
| y | The data to be compared among the levels of the grouping factor |

### Value

A list with three matrices: 1) A matrix with the number of overlapping data; 2) A matrix with the percentage of overlapping; 3) A matrix with the combination of the two previous one

### Examples

```
data(QTLmarkers)
data(gtfGenes)
genes.out <- find_genes_qtls_around_markers(db_file=gtfGenes,
marker_file=QTLmarkers,method="gene",
marker="snp",interval=100000, nThreads=1)
overlapping.out<-overlapping_among_groups(
file=genes.out,x="Reference",y="gene_id")
```

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PleioChiTest *Compute a multi-trait test statistic for pleiotropic effects using summary statistics from association tests*

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### Description

Compute a multi-trait test statistic for pleiotropic effects using summary statistics from association tests

### Usage

```
PleioChiTest(data)
```

**Arguments**

data          A data frame with the first column containing the SNP name and the remaining columns the signed t-values obtained for each marker in the association studies individually performed for each trait.

**Details**

This function tests a null hypothesis stating that each SNP does not affect any of the traits included in the input file. The method applied here is an implementation of the statistic proposed at Bolormaa et al. (2014) and is approximately distributed as a chi-squared with n degrees of freedom, where n is equal the number of traits included in the input file.

**Value**

A data frame with the multi-trait chi-squared statistics and the correspondent p-value obtained for each SNP.

**References**

Bolormaa et al. (2014) Plos Genetics, Volume 10, Issue 3, e1004198. (doi:10.1371/journal.pgen.1004198)

---

plot_overlapping        *Plot overlapping between data and grouping factors*

---

**Description**

Takes the output from overlapping_among_groups function and creates a heatmap with the overlapping between groups

**Usage**

```
plot_overlapping(overlapping_matrix, nmatrix, ntext, group, labelcex = 1)
```

**Arguments**

overlapping_matrix

         The object obtained in overlapping_amoung_groups function

nmatrix       An interger from 1 to 3 indicating which matrix will be used to plot the overlapping, where: 1) A matrix with the number of overllaping data; 2) A matrix with the percentage of overlapping; 3) A matrix with the combination of the two previous one

ntext         An interger from 1 to 3 indicating which matrix will be used as the text matrix for the heatmap, where: 1) A matrix with the number of overllaping data; 2) A matrix with the percentage of overlapping; 3) A matrix with the combination of the two previous one

group         A vector with the size of groups. This vector will be plotted as row and column names in the heatmap

labelcex       A numeric value indicating the size of the row and column labels

## Value

A heatmap with the overlapping between groups

## Examples

```
data(QTLmarkers)
data(gtfGenes)
genes.out <- find_genes_qtls_around_markers(
db_file=gtfGenes, marker_file=QTLmarkers,
method="gene", marker="snp",interval=100000,
nThreads=1)

overlapping.out<-overlapping_among_groups(
file=genes.out,x="Reference",y="gene_id")
plot_overlapping(overlapping.out,
nmatrix=2,ntext=2,
group=unique(genes.out$Reference))
```

---

| plot_qtl_info | *Plot QTLs information from the find_genes_qtls_around_markers output* |
|---|---|

---

## Description

Takes the output from find_genes_qtls_around_markers and create plots for the frequency of each QTL type and trait

## Usage

```
plot_qtl_info(
  qtl_file,
  qtl_plot = c("qtl_type", "qtl_name"),
  n = "all",
  qtl_class = NULL,
  horiz = FALSE,
  ...
)
```

## Arguments

| | |
|---|---|
| `qtl_file` | The output from find_genes_qtls_around_markers function |
| `qtl_plot` | "qtl_type" or"qtl_name" |
| `n` | Number of QTLs to be plotted when the qtl_name option is selected |
| `qtl_class` | Class of QTLs to be plotted when the qtl_name option is selected |
| `horiz` | The legend of the pie plot for the qtl_type should be plotted vertically or horizontally. The default is FALSE. Therefore, the legend is plotted vertically. |

    ...                   Arguments to be passed to/from other methods. For the default method these can include further arguments (such as axes, asp and main) and graphical parameters (see par) which are passed to plot.window(), title() and axis.

## Value

A plot with the requested information

## Examples

```
data(QTLmarkers)
data(gffQTLs)

out.qtls<-find_genes_qtls_around_markers(db_file=gffQTLs,
marker_file=QTLmarkers, method = "qtl",
marker = "snp", interval = 500000,
nThreads = 1)

plot_qtl_info(out.qtls, qtl_plot = "qtl_type", cex=2)
```

---

QTLenrich_plot               *Plot enrichment results for QTL enrichment analysis*

---

## Description

Takes the output from qtl_enrich function and creates a bubble plot with enrichment results

## Usage

```
QTLenrich_plot(qtl_enrich, x, pval)
```

## Arguments

    qtl_enrich      The output from qtl_enrich function

    x                Id column to be used from the qtl_enrich output

    pval            P-value to be used in the plot. The name informed to this argument must match the p-value column name in the enrichment table

## Value

A plot with the QTL enrichment results

| qtl_enrich | *Performs a QTL enrichment analysis based on a hypergeometric test for each QTL class* |
|---|---|

## Description

Takes the output from find_genes_qtls_around_markers and run a QTL enrichment analysis

## Usage

```
qtl_enrich(
  qtl_db,
  qtl_file,
  qtl_type = c("QTL_type", "Name"),
  enrich_type = c("genome", "chromosome"),
  chr.subset = NULL,
  nThreads = NULL,
 padj = c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none"),
  verbose = TRUE
)
```

## Arguments

| | |
|---|---|
| qtl_db | The object obtained using the import_gff_gtf() function |
| qtl_file | The output from find_genes_qtls_around_markers function |
| qtl_type | A character indicating which type of enrichment will be performed. QTL_type indicates that the enrichment processes will be performed for the QTL classes, while Name indicates that the enrichment analysis will be performed for each trait individually |
| enrich_type | A character indicating if the enrichment analysis will be performed for all the chromosomes ("genome") or for a subset of chromosomes ("chromosome"). If the "genome" option is selected, the results reported are the merge of all chromosomes |
| chr.subset | If enrich_type equal "chromosome", it is possible to define a subset of chromosomes to be analyzed. The default is equal NULL. Therefore, all the chromosomes will be analyzed |
| nThreads | The number of threads to be used. |
| padj | The algorithm for multiple testing correction to be adopted ("holm", "hochberg", "hommel", "bonferroni", "BH", "BY","fdr", "none") |
| verbose | Logical value defining if messages should of not be printed during the analysis (default=TRUE) |

**Details**

The simple bias of investigation for some traits (such as milk production related traits in the QTL database for cattle) may result in a larger proportion of records in the database. Consequently, the simple investigation of the proportion of each QTL type might not be totally useful. In order to reduce the impact of this bias, a QTL enrichment analysis can be performed. The QTL enrichment analysis performed by GALLO package is based in a hypergeometric test using the number of annoatted QTLs within the candidate regions and the total number of the same QTL in the QTL database.

**Value**

A data frame with the p-value for the enrichment result

**Examples**

```
data(QTLmarkers)
data(gffQTLs)
out.qtls<-find_genes_qtls_around_markers(
db_file=gffQTLs,marker_file=QTLmarkers,
method = "qtl",marker = "snp",
interval = 500000, nThreads = 1)

out.enrich<-qtl_enrich(qtl_db=gffQTLs,
qtl_file=out.qtls, qtl_type = "Name",
enrich_type = "chromosome",chr.subset = NULL,
padj = "fdr",nThreads = 1)
```

---

relationship_plot          *Plot relationship between data and grouping factors*

---

**Description**

Takes the output from find_genes_qtls_around_markers function and creates a chord plot with the relationship between groups

**Usage**

```
relationship_plot(
  qtl_file,
  x,
  y,
  grid.col = "gray60",
  degree = 90,
  canvas.xlim = c(-2, 2),
  canvas.ylim = c(-2, 2),
  cex,
  gap
)
```

## Arguments

| | |
|---|---|
| qtl_file | The output from find_genes_qtls_around_markers function |
| x | The first grouping factor, to be plotted in the left hand side of the chord plot |
| y | The second grouping factor, to be plotted in the left hand side of the chord plot |
| grid.col | A character with the grid color for the chord plot or a vector with different colors to be used in the grid colors. Note that when a color vector is provided, the length of this vector must be equal the number of sectors in the chord plot |
| degree | A numeric value corresponding to the starting degree from which the circle begins to draw. Note this degree is always reverse-clockwise |
| canvas.xlim | The coordinate for the canvas in the x-axis. By default is c(-1,1) |
| canvas.ylim | The coordinate for the canvas in the y-axis. By default is c(-1,1) |
| cex | The size of the labels to be printed in the plot |
| gap | A numeric value corresponding to the gap between the chord sectors |

## Value

A chords relating x and y

## Examples

```
data(QTLmarkers)
data(gffQTLs)
out.qtls<-find_genes_qtls_around_markers(
db_file=gffQTLs, marker_file=QTLmarkers,
method = "qtl", marker = "snp",
interval = 500000, nThreads = 1)

out.enrich<-qtl_enrich(qtl_db=gffQTLs,
qtl_file=out.qtls, qtl_type = "Name",
enrich_type = "chromosome",
chr.subset = NULL, padj = "fdr",nThreads = 1)

out.enrich$ID<-paste(out.enrich$QTL," - ",
"CHR",out.enrich$CHR,sep="")

out.enrich.filtered<-out.enrich[which(out.enrich$adj.pval<0.05),]

out.qtls$ID<-paste(out.qtls$Name," - ",
"CHR",out.qtls$CHR,sep="")

out.enrich.filtered<-out.enrich.filtered[order(out.enrich.filtered$adj.pval),]

out.qtls.filtered<-out.qtls[which(out.qtls$ID%in%out.enrich.filtered$ID[1:10]),]

out.qtls.filtered[which(out.qtls.filtered$Reference==
"Feugang et al. (2010)"), "color_ref"]<-"purple"

out.qtls.filtered[which(out.qtls.filtered$Reference==
```

```
"Buzanskas et al. (2017)"),"color_ref"]<-"pink"

color.grid<-c(rep("black",length(unique(out.qtls.filtered$Abbrev))),
unique(out.qtls.filtered$color_ref))

names(color.grid)<-c(unique(out.qtls.filtered$Abbrev),
unique(out.qtls.filtered$Reference))

relationship_plot(qtl_file=out.qtls.filtered,
x="Abbrev", y="Reference",cex=1,gap=5,
degree = 90, canvas.xlim = c(-5, 5),
canvas.ylim = c(-3, 3), grid.col = color.grid)
```

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