

Package ‘trigger’

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ExpRession

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Imports qvalue, methods, graphics, sva

Description This R package provides tools for the statistical analysis of integrative genomic data that involve some combination of: genotypes, high-dimensional intermediate traits (e.g., gene expression, protein abundance), and higher-order traits (phenotypes). The package includes functions to: (1) construct global linkage maps between genetic markers and gene expression; (2) analyze multiple-locus linkage (epistasis) for gene expression; (3) quantify the proportion of genome-wide variation explained by each locus and identify eQTL hotspots; (4) estimate pair-wise causal gene regulatory probabilities and construct gene regulatory networks; and (5) identify causal genes for a quantitative trait of interest.

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plot	<i>Graphical Display of Trigger Analysis</i>
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Description

Graphical display of genomewide linkage map, multi-locus linkage or eQTL variation

Usage

```
## S4 method for signature 'trigger,missing'
plot(x,y,type = c("link", "mlink", "eqtl"),
     cutoff = 3.3e-4, qcut = 0.1, bin.size = NULL)
```

Arguments

x	An object of class <code>trigger</code> .
y	Ignore option, not used.
type	An argument describing the type of plot. Select from <code>link</code> (default) for genome-wide linkage map, <code>eqtl.R2</code> for graphical display of eQTL-R ² contribution or <code>mlink</code> for display of genome-wide epistasis effect.
cutoff	Threshold value for <code>link</code> . The measures below the threshold are called significant and are plotted.
qcut	Q-value threshold for <code>mlink</code> . The joint multi-locus linkage probabilities with q-values below the threshold are called significant and are plotted.
bin.size	Optional for <code>mlink</code> . If not <code>NULL</code> , each chromosome will be divided into several bins, each with size <code>bin.size</code> . Markers within a bin will be considered as at a same position.

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and John D. Storey <jstorey@princeton.edu>

See Also

[trigger.link](#), [trigger.mlink](#) and [trigger.eigenR2](#)

Examples

```
## Not run:

data(yeast)
attach(yeast)
triggerobj <- trigger.build(marker=marker, exp=exp,
marker.pos=marker.pos, exp.pos=exp.pos)
triggerobj <- trigger.link(triggerobj, gender=NULL, norm=TRUE)
plot(triggerobj,type = "link", cutoff=1e-5)
triggerobj <- trigger.eigenR2(triggerobj, adjust=FALSE)
plot(triggerobj, type = "eigenR2")
triggerobj<- trigger.mlink(triggerobj, B=5, seed=123)
plot(triggerobj, qcut=0.1, bin.size=NULL)

detach(yeast)

## End(Not run)
```

trigger-class

A class to store and analyze data for Transcriptional Regulation Inference from Genetics of Gene Expression

Description

trigger is a class of objects to store and analyze data for Integrative Genomic Analysis. Use [trigger.build](#) to generate new objects of the class from input data.

Details

The positions in marker.pos and exp.pos matrix should be in the same units (e.g., base pair, kb, or cM).

Value

An object of S4 class [trigger](#) containing the marker genotype matrix (a matrix of 1,2 for haploid genotypes, or 1,2,3 for diploid genotypes), expression matrix, marker position matrix and gene/trait position matrix with ordered coordinates in respective slots. Use `slot(objectname, varname)` to retrieve individual variables from the object. Use `print` to see the first 10 rows and columns of the expression and marker matrix.

Slots

- exp:** A numeric matrix with *m* rows and *n* columns, containing the gene expression (or intermediate trait) data.
- exp.pos:** A matrix with *m* rows and 3 columns containing the chromosome number, gene start and gene end for all the genes in the gene expression matrix. The rows of `exp.pos` should match those of `exp`.
- marker:** A matrix with *p* rows and *n* columns, containing genotyping information.
- marker.pos:** A matrix with *p* rows and 2 columns containing the chromosome number and SNP position for all the genes in the gene expression matrix. The rows of `exp.pos` should match those of `exp`.
- stat:** A matrix of pair-wise likelihood ratio statistics for linkage analysis, with genes in rows and markers in columns.
- pvalue:** A matrix of parametric pvalues corresponding to statistics in the `stat` matrix.
- mlink:** A list containing the results of Multi-locus linkage analysis. See [trigger.mlink](#) for details.
- eqtl.R2:** A vector containing the proportion of genome-wide variation explained by each observed locus (eQTL). See [trigger.eigenR2](#) for details.
- loc.obj:** A list containing the results of local-linkage probability estimation. See [trigger.loclink](#) for details.

Author(s)

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See Also

[trigger.build](#), [trigger.link](#), [trigger.mlink](#), [trigger.eigenR2](#), [trigger.net](#) and [trigger.trait](#)

trigger.build

Format the input data and create an Trigger object

Description

This function takes high-dimensional expression data and genotype data with each of their position data in the genome and creates a [trigger](#) object for subsequent analysis.

Usage

```
trigger.build(exp = exp, exp.pos = exp.pos, marker = marker, marker.pos = marker.pos)
```

Arguments

exp	A gene (or intermediate trait) by individual matrix of expression data.
exp.pos	A matrix containing the position information for genes (intermediate traits). The first column is the chromosome name of the gene. The second column is the starting coordinate of the gene, and the third column is the ending coordinate. Each row corresponds to one gene/trait in the exp matrix.
marker	A marker genotype by individual matrix.
marker.pos	A matrix containing the position information for markers. The first column is the chromosome name of the marker. We recommend to use integers for autosomal chromosomes and "X" for sex chromosome. The second column is the position of the marker on the chromosome. Each row corresponds to one marker in the marker matrix.

Details

The positions in marker.pos and exp.pos matrix should be in the same units (e.g., base pair, kb, or cM).

Value

An object of S4 class `trigger` containing the marker genotype matrix (a matrix of 1,2 for haploid genotypes, or 1,2,3 for diploid genotypes), expression matrix, marker position matrix and gene/trait position matrix with ordered coordinates in respective slots. Use `slot(objectname, varname)` to retrieve individual variables from the object. Use `print` to see the first 10 rows and columns of the expression and marker matrix.

Author(s)

Lin S. Chen <lschen.stat@gmail.com>, Dipen P. Sangurdekar <dps@genomics.princeton.edu> and John D. Storey <jstorey@princeton.edu>

See Also

[trigger.link](#), [trigger.mlink](#), [trigger.eigenR2](#), [trigger.net](#) and [trigger.trait](#)

Examples

```
## Not run:
data(yeast)
attach(yeast)
triggerobj <- trigger.build(marker = marker, exp = exp,
                           marker.pos = marker.pos, exp.pos = exp.pos)
print(triggerobj)

## End(Not run)
```

trigger.eigenR2-methods

Estimate the proportion of genome-wide variation explained by each eQTL

Description

Estimate eqtl-R2, the proportion of genome-wide variation explained by each eQTL and identify linkage hotspots.

Usage

```
## S4 method for signature 'trigger'  
trigger.eigenR2(triggerobj, adjust = FALSE, meanR2 = FALSE)
```

Arguments

triggerobj	An object of class <code>trigger</code> .
adjust	Logical. If TRUE, the estimated R-square for each locus will be adjusted for small sample size effect. Recommend to use when sample size is less than 100.
meanR2	Logical. If TRUE, the function computes the mean of R-squares of genome-wide gene expression for each locus.

Value

An updated object of class `trigger` with a slot `loc.obj` containing the proportion of genome-wide variation explained by each observed locus (eQTL). Use `slot(triggerobj, "eigenR2")` to retrieve the eqtl-R2 values as a vector.

Author(s)

Lin S. Chen <lschen.stat@gmail.com>, Dipen P. Sangurdekar <dps@genomics.princeton.edu> and John D. Storey <jstorey@princeton.edu>

References

Chen L.S. and Storey J.D. (2008) Eigen-R2 for dissecting variation in high-dimensional studies. *Bioinformatics* **24(19)**: 2260–2262.

See Also

`plot`

Examples

```
## Not run:
  data(yeast)
  attach(yeast)
  triggerobj <- trigger.build(marker = marker, exp = exp,
marker.pos = marker.pos, exp.pos = exp.pos)
  triggerobj <- trigger.eigenR2(triggerobj, adjust = FALSE)
  plot(triggerobj, type = "eigenR2")
  eqtlR2 <- slot(triggerobj, "eigenR2")
  detach(yeast)

## End(Not run)
```

trigger.export2cross-methods

Export Trigger data to R/qt1's cross class object

Description

trigger.export2cross exports [trigger](#) data from triggerobj to a cross format for Trait-Triple analysis. See [trigger.trait](#) for details.

Usage

```
## S4 method for signature 'trigger'
trigger.export2cross(triggerobj, plotarg = TRUE, verbose = TRUE, warning = FALSE)
```

Arguments

triggerobj	An object of class trigger .
plotarg	Logical. If TRUE, the function plots the default plot from the R/qt1 package while reading in the genotype data.
verbose	Logical. If TRUE, the function lists the default output from the R/qt1 package while reading in the genotype data.
warning	Logical. If FALSE, the function suppresses warnings output from the R/qt1 package while reading in the genotype data.

Details

The trigger.export2cross command writes a csv format file “geno_trait_data.csv” to the working directory and reads it using the read.cross command.

Value

An object of class cross from the R/qt1 package.

Author(s)

Lin S. Chen <lschen.stat@gmail.com>, Dipen P. Sangurdekar <dps@genomics.princeton.edu>
and John D. Storey <jstorey@princeton.edu>

References

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics* **19**: 889–890.

See Also

[trigger.trait](#)

Examples

```
data(yeast)
attach(yeast)
triggerobj <- trigger.build(marker = marker, exp = exp,
marker.pos = marker.pos, exp.pos = exp.pos)
crossfile <- trigger.export2cross(triggerobj, plotarg = TRUE, verbose = TRUE, warning = FALSE)
tt.pval <- trigger.trait(triggerobj, trait = "DSE1", cross = crossfile)
causal.reg <- names(which(p.adjust(tt.pval, method = "fdr") < .05))
detach(yeast)
```

trigger.link-methods *Genomewide eQTL analysis*

Description

A method of class [trigger](#) for genomewide Expression-trait QTL analysis. This function estimates the linkage statistic and parametric p-value for each gene expression to every locus in the genome.

Usage

```
## S4 method for signature 'trigger'
trigger.link(triggerobj, gender = NULL, norm = TRUE)
```

Arguments

triggerobj	An object of class trigger .
gender	Optional. When computing linkage statistics involving markers on sex chromosome, gender of each sample should be specified.
norm	Logical. If TRUE, each row of expression matrix exp in the triggerobj will be transformed to follow a standard normal distribution, based on the rank of value.

Value

An updated object of class `trigger` containing slots:

`stat` A matrix of pair-wise likelihood ratio statistics for linkage analysis, with genes in rows and markers in columns.

`pvalue` A matrix of parametric pvalues corresponding to statistics in the `stat` matrix.

Use `slot(triggerobj, "stat")` and `slot(triggerobj, "pvalue")` to retrieve the values.

Author(s)

Lin S. Chen <lschen.stat@gmail.com>, Dipen P. Sangurdekar <dps@genomics.princeton.edu> and John D. Storey <jstorey@princeton.edu>

See Also

`plot` and `trigger.mlink`

Examples

```
## Not run:
  data(yeast)
  attach(yeast)
  triggerobj <- trigger.build(marker = marker, exp = exp,
marker.pos = marker.pos, exp.pos = exp.pos)
  triggerobj <- trigger.link(triggerobj, gender = NULL, norm = TRUE)
  plot(triggerobj, type = "link", cutoff = 1e-5)
  stat = slot(triggerobj, "stat"); pvalue = slot(triggerobj, "pvalue")
  detach(yeast)

## End(Not run)
```

trigger.loclink-methods

Estimate local-linkage probability for each gene

Description

A method of class `trigger` to identify the best local-linkage marker for each gene and compute the local linkage probabilities.

Usage

```
## S4 method for signature 'trigger'
trigger.loclink(triggerobj, gender = NULL, window.size = 30000)
```

Arguments

triggerobj	An object of class <code>trigger</code> .
gender	Optional. When computing linkage statistics involving markers on sex chromosome, gender of each sample should be specified.
window.size	Optional. The size of a window that places the putative regulator gene in the center. Every marker within the window is a candidate marker for local-linkage to the regulator gene.

Value

An updated object of class `trigger` containing a slot `loc.obj` with fields:

prob.loc	The estimated local-linkage probability for each putative regulator gene.
loc.idx	The indices of the best local marker for each putative regulator gene.

Use `slot(triggerobj, "loc.obj")` to retrieve the list.

Author(s)

Lin S. Chen <lschen.stat@gmail.com>, Dipen P. Sangurdekar <dps@genomics.princeton.edu> and John D. Storey <jstorey@princeton.edu>

References

Chen L.S., Emmert-Streib F., and Storey J.D. (2007) Harnessing naturally randomized transcription to infer regulatory relationships among genes. *Genome Biology*, **8**: R219.

See Also

[trigger.trait](#)

Examples

```
## Not run:
data(yeast)
attach(yeast)
triggerobj <- trigger.build(marker = marker, exp = exp,
                           marker.pos = marker.pos, exp.pos = exp.pos)
triggerobj <- trigger.loclink(triggerobj, window.size = 30000)
trigger.obj <- trigger.net(triggerobj, Bsec = 100)
detach(yeast)

## End(Not run)
```

`trigger.mlink-methods` *Multi-Locus Linkage (Epistasis) Analysis*

Description

Multi-locus linkage (epistasis) analysis.

Usage

```
## S4 method for signature 'trigger'  
trigger.mlink(triggerobj, prob.cut = 0.9,  
gender = NULL, idx = NULL, B = 5, seed = 123)
```

Arguments

<code>triggerobj</code>	An object of class <code>trigger</code> .
<code>prob.cut</code>	Probability threshold for primary linkage.
<code>gender</code>	Optional. When computing linkage statistics involving markers on sex chromosome, gender of each sample should be specified.
<code>idx</code>	The indices for genes to be computed for multi-locus linkage.
<code>B</code>	The number of null iterations to perform.
<code>seed</code>	Optional. A numeric seed for reproducible results.

Details

When data set is large, one can the option `idx` to select a subset of genes in each computation and parallel-computes the genome-wide multi-locus linkage. Since the function computes the linkage probability by borrowing information across genes, at least more than 100 genes should be selected in applying this function. If `idx=NULL`, all the genes in the input data will be computed for multi-locus linkage.

The current version of the function could only compute two-locus joint linkage (epistasis).

Value

An updated object of class `trigger` containing a slot `trigger.mlink` with fields:

<code>qt1</code>	The major and secondary QTLs for each selected gene.
<code>prob</code>	The posterior probability of linkage for major QTL, secondary QTL, and the joint posterior probability of multi-locus linkage.
<code>qvalue</code>	Q-value estimates for joint multi-locus linkage probabilities.

Use `slot(triggerobj, "mlink")` to retrieve the list.

Author(s)

Lin S. Chen <lschen.stat@gmail.com>, Dipen P. Sangurdekar <dps@genomics.princeton.edu>
and John D. Storey <jstorey@princeton.edu>

References

Brem R.B., Storey J.D., Whittle J., and Kruglyak L. (2005) Genetic interactions between polymorphisms that affect gene expression in yeast. *Nature*, **436(7051)**: 701–703.

Storey J.D., Akey J.M., and Kruglyak L. (2005) Multiple locus linkage analysis of genomewide expression in yeast. *PLoS Biology*, **3(8)**: 1380–1390.

See Also

[trigger.link](#) and [plot](#)

Examples

```
## Not run:
data(yeast)
  attach(yeast)
triggerobj <- trigger.build(marker = marker, exp = exp,
  marker.pos = marker.pos, exp.pos = exp.pos)
## Genome-wide multiple locus linkage analysis
triggerobj <- trigger.mlink(triggerobj, B = 10, idx = NULL, seed = 123)

plot(triggerobj, type = "trigger.mlink", qcut=0.1, bin.size=NULL)
mlink = slot(triggerobj, "trigger.mlink")
detach(yeast)

## End(Not run)
```

trigger.net-methods *Network-Trigger analysis*

Description

Network-Trigger analysis estimates the joint posterior probability of causal regulation for each pair of genes in the genome. These probabilities can further be used to construct a gene regulatory network.

Usage

```
## S4 method for signature 'trigger'
trigger.net(triggerobj, gender = NULL, idx = NULL,
  Bsec = 100, prob.cut = 0.7, include.loc = TRUE, seed = 123, inputfile = NULL)
```

Arguments

triggerobj	An object of class trigger containing slot loc.obj with local-linkage probabilities and marker indices of the best local-linkage markers for genes in the genome. See trigger and trigger.loclink for details.
gender	Optional. When computing statistics involving markers on sex chromosome, gender of each sample should be specified.

idx	Optional. One can specify the indices of selected genes as putative regulators. By default, all the genes will be selected as putative regulators.
Bsec	Number of iterations to perform when estimating null statistics for secondary-linkage and conditional independence.
prob.cut	Probability threshold. The joint regulatory probabilities of a regulator to all the other genes will be set to zero if the local-linkage probability of the regulator is below the threshold; default prob.cut = 0.7.
include.loc	Logical. If TRUE, the estimated posterior probability of regulation is more conservative.
seed	Optional. A numeric seed for reproducible results.
inputfile	Optional. If provided, reads in the probability matrix from working directory.

Details

The option `idx` contains the indices of putative regulator genes. When the data set is large, one can use this option by selecting a subset of genes as putative regulators in one computation and parallel-computes the genome-wide regulatory probability. If `idx=NULL`, all the genes will be computed for probability of regulation to other genes in the data.

If `include.loc = TRUE`, the joint posterior probability of regulation is the product of local-linkage, secondary-linkage and conditional independence. Otherwise, it is the product of secondary-linkage and conditional independence. The local-linkage is not a necessary condition for calculating regulation probability. If the probability of local-linkage is considered, the joint probability of regulation is more conservative. See references for details.

Value

A matrix of genome-wide regulatory probabilities with putative regulators in rows and regulated genes in columns. Note that the matrix is not symmetric. If gene *i* is estimated to be causal for gene *j* with high probability, the reverse is not true.

Author(s)

Lin S. Chen <lschen.stat@gmail.com>, Dipen P. Sangurdekar <dps@genomics.princeton.edu> and John D. Storey <jstorey@princeton.edu>

References

Chen L.S., Emmert-Streib F., and Storey J.D. (2007) Harnessing naturally randomized transcription to infer regulatory relationships among genes. *Genome Biology*, **8**: R219.

See Also

[trigger.loclink](#), [trigger.netPlot2ps](#) and [trigger.trait](#)

Examples

```
## Not run:
data(yeast)
attach(yeast)
triggerobj <- trigger.build(marker = marker, exp = exp,
                           marker.pos = marker.pos, exp.pos = exp.pos)
triggerobj <- nettrig.loc(triggerobj, window.size = 30000)
trig.prob <- trigger.net(triggerobj, Bsec = 100)
netPlot2ps(trig.prob)
detach(yeast)

## End(Not run)
```

```
trigger.netPlot2ps-methods
```

Write the network from a trigger probability matrix to a postscript file

Description

Write the network from a trigger probability matrix to a postscript file.

Usage

```
## S4 method for signature 'trigger'
trigger.netPlot2ps(triggerobj, trig.prob, filenam = NULL, pcut = 0.95,
                  layout = c("radial", "energy-minimized", "circular", "hierarchical"),
                  node.color = NULL, edge.color = NULL, node.shape = NULL, nreg = 20)
```

Arguments

triggerobj	An object of class <code>trigger</code> .
trig.prob	A network-Trigger regulatory probability matrix with putative regulator genes in rows and putative regulated genes in columns. See trigger.net for details.
filenam	The output file name, without extension. If the name is not specified, the network will be write to the files <code>temp.ps</code> and <code>temp.dot</code> at the current directory.
pcut	Threshold value for regulatory probabilities. The probabilities above the threshold are called significant and the corresponding regulatory relationships are plotted.
layout	The layout of the output network. One can choose from "radial" (default), "energy-minimized", "circular" or "hierarchical" layouts. You can specify just the initial letter.
node.color	The color of the nodes (genes). The default color is green.
edge.color	The color of the edges. The default color is blue.
node.shape	The shape of nodes (genes) if the number of regulatory relationships is below 1000. If that number is above 1000, the shape of nodes will be dot.
nreg	The number of top regulators to be selected. These selected top regulators will be plotted in red ellipses with their gene names labeled inside.

Details

To use this function, please install the software Graphviz, which is available at <http://www.graphviz.org/>. For large networks, layout "radial" or "energy-minimized" is recommended. If the total number of significant regulatory relationships (directed edges) of the network is below 1000, we plot each node (gene) as a "box" with its name labeled inside. Otherwise, we plot each gene as a "dot" without name labeled to facilitate visualization. The top nreg (by default nreg = 20) regulators will be plotted in red ellipses labeled with their names.

See manual of Graphviz for other available colors and shapes of nodes.

Author(s)

Lin S. Chen <lschen.stat@gmail.com>, Dipen P. Sangurdekar <dps@genomics.princeton.edu> and John D. Storey <jstorey@princeton.edu>

See Also

[trigger.link](#) and [trigger.mlink](#)

Examples

```
## Not run:
data(yeast)
attach(yeast)
triggerobj <- trigger.build(marker = marker, exp = exp,
marker.pos = marker.pos, exp.pos = exp.pos)
triggerobj <- trigger.loclink(triggerobj, window.size = 30000)
trig.prob <- trigger.net(triggerobj, Bsec = 100)

trigger.netPlot2ps(trig.prob, pcut = 0.95, layout = "e", filenam = "net95", nreg = 20)

detach(yeast)

## End(Not run)
```

trigger.trait-methods *Trait-trigger analysis*

Description

Trait-Trigger identifies, for a given trait of interest, causal gene regulator(s) that makes the trait conditionally independent of the QTL and their estimated p-values of causal regulation. These probabilities can further be used to construct a gene regulatory network.

Usage

```
## S4 method for signature 'trigger'
trigger.trait(triggerobj, trait, cross, thr, n.sv = NULL, addplot = TRUE)
```

Arguments

triggerobj	An object of class <code>trigger</code> . See <code>trigger</code> for details.
trait	Trait for which causal regulator is to be found. It can either be a gene-name for a gene expression trait present in <code>triggerobj</code> or a vector of values for the individuals present in <code>triggerobj</code> .
cross	An object of class <code>cross</code> obtained from <code>trigger.export2cross</code> . See <code>R/qt1</code> for more details.
thr	LOD threshold to search for locally linked putative causal genes (default 3).
n.sv	Number of surrogate variables used to model the local heterogeneity. If not set, it is computed from the expression data.
addplot	If TRUE, a plot of the LOD scores from a genome-scan for a single-QTL model from package <code>R/qt1</code> .

Value

A vector of p-values associated with each tested causal regulator.

Author(s)

Lin S. Chen <lschen.stat@gmail.com>, Dipen P. Sangurdekar <dps@genomics.princeton.edu> and John D. Storey <jstorey@princeton.edu>

References

- Chen L.S., Emmert-Streib F., and Storey J.D. (2007) Harnessing naturally randomized transcription to infer regulatory relationships among genes. *Genome Biology*, **8**: R219.
- Broman KW, Wu H, Sen S, Churchill GA (2003) `R/qt1`: QTL mapping in experimental crosses. *Bioinformatics* **19**: 889–890.

See Also

`trigger.loclink` and `trigger.export2cross`

Examples

```
## Not run:
data(yeast)
attach(yeast)
triggerobj <- trigger.build(marker = marker, exp = exp,
  marker.pos = marker.pos, exp.pos = exp.pos)
crossfile <- trigger.export2cross(triggerobj)
tt.pval <- trigger.trait(triggerobj, trait = "DSE1", cross = crossfile)
causal.reg <- names(which(p.adjust(tt.pval, method = "fdr") < .05))
detach(yeast)

## End(Not run)
```

yeast-data

A yeast data set for Transcriptional Regulation Inference from Genetics of Gene Expression

Description

A yeast data set for integrative genomic analysis.

Details

The data set contains information on 112 F1 segregants from a yeast genetic cross of BY and RM strains. The list consists of: `marker`: A 3244 x 112 genotype matrix with marker genotypes in rows and arrays in columns. `exp`: A 6216 x 112 gene expression matrix with genes in rows and arrays in columns. `marker.pos`: A matrix of marker position information. `exp.pos`: A matrix of gene position information.

References

Brem R.B., Storey J.D., Whittle J., and Kruglyak L. (2005) Genetic interactions between polymorphisms that affect gene expression in yeast. *Nature*, **436(7051)**: 701–703.

Storey J.D., Akey J.M., and Kruglyak L. (2005) Multiple locus linkage analysis of genomewide expression in yeast. *PLoS Biology*, **3(8)**: 1380–1390.

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