Package ‘gemini’

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Type Package

Title GEMINI: Variational inference approach to infer genetic interactions from pairwise CRISPR screens

Version 1.18.0

Description GEMINI uses log-fold changes to model sample-dependent and independent effects, and uses a variational Bayes approach to infer these effects. The inferred effects are used to score and identify genetic interactions, such as lethality and recovery. More details can be found in Zamanighomi et al. 2019 (in press).

Depends R (>= 4.1.0)

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BugReports https://github.com/sellerslab/gemini/issues

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Suggests knitr, rmarkdown, testthat

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Description

Median normalization

Usage

.median_normalize(counts, CONSTANT = 32)

Arguments

counts a counts matrix derived from Input
CONSTANT a pseudo-count to use (default = 32)
Value

median-normalized counts object

Examples

```r
## Not run:
.median_normalize(rnorm(n = 1000))
## End(Not run)
```

Counts matrix from the Big Papi SynLet dataset. The Big Papi dataset was published in Najm et al. 2018 (doi: 10.1038/nbt.4048). The counts were acquired from Supplementary Table 3.

Usage

counts

Format

An object of class `matrix` (inherits from `array`) with 9216 rows and 16 columns.

See Also

https://www.nature.com/articles/nbt.4048

gemini_boxplot

description

A function to visualize the results of GEMINI over the raw data.

Usage

gemini_boxplot(
  Model,
  g,
  h,
  nc_gene = NULL,
  sample,
  show_inference = TRUE,
  color_x = FALSE,
  identify_guides = FALSE
)
Arguments

Model  a gemini.model object

g  a character naming a gene to visualize

h  a character naming another gene to visualize

nc_gene  a character naming the gene to use as a negative control, to be paired with each individual g and h. Defaults to Model$nc_gene.

sample  a character naming the sample to visualize

show_inference  a logical indicating whether to show the inferred individual or combined values for each gene/gene pair (default TRUE)

color_x  a logical indicating whether to visualize the sample-independent effects for each individual guide or guide pair (default FALSE)

identify_guides  a logical indicating whether to identify guides with unique colors and shapes (default FALSE)

Details

Raw LFC data is plotted for each gene combination ('g'-'nc_gene', 'h'-'nc_gene', 'g'-'h') in a standard boxplot. Horizontal green line segments are plotted over the box plots indicating the individual gene effects or the inferred total effect of a particular gene combination. Each guide pair can be colored based on the inferred sample independent effects \( g_i, h_j \), and \( g_i, h_j \). Additionally, colors and shapes can be used to distinguish unique guides targeting gene g and h, respectively.

Value

a ggplot2 object

Examples

data("Model", package = "gemini")

gemini_boxplot(Model, g = "BRCA2", h = "PARP1", nc_gene = "CD81", sample = "A549", show_inference = TRUE, color_x = FALSE, identify_guides = FALSE)

---

**gemini_calculate_lfc**  *Calculate log-fold change*

Description

Given a gemini.input object, calculates log-fold change from counts.
Usage

```r
gemini_calculate_lfc(Input, counts = "counts", sample.column.name = "samplename", normalize = TRUE, CONSTANT = 32)
```

Arguments

- **Input**: a gemini.input object containing an object named `counts`.
- **counts**: a character indicating the name of a matrix of counts within `Input` that can be used to calculate log-fold changes (defaults to "counts").
- **sample.column.name**: a character or integer indicating which column of `Input$replicate.map` describes the samples.
- **normalize**: a logical indicating if counts should be median-normalized, see Details (default = TRUE)
- **CONSTANT**: a numeric indicating a constant value that shifts counts to reduce outliers, see Details (default = 32).

Details

See Methods from Zamanighomi et al. 2019 for a comprehensive description of the calculation of log-fold change, normalization, and count processing.

If multiple early time-points are provided for a given sample, they are treated as replicates and averaged, and used to compute log-fold change against any specified late time points.

If a sample has a specific early-time point, these are matched as long as the sample names are identical between the early and late timepoints in `sample.column.name`.

Value

a gemini object identical to `Input` that also contains new objects called LFC and sample.annot.

Examples

```r
data("Input", package = "gemini")
Input <- gemini_calculate_lfc(Input)

head(Input$LFC)
```

Description

Creates a gemini.input object from a counts matrix with given annotations.
Usage

```r
gemini_create_input(
  counts.matrix,
  sample.replicate.annotation = NULL,
  guide.annotation = NULL,
  samplesAreColumns = TRUE,
  sample.column.name = "samplename",
  gene.column.names = NULL,
  ETP.column = 1,
  LTP.column = NULL,
  verbose = FALSE
)
```

Arguments

- `counts.matrix`: a matrix of counts with rownames corresponding to features (e.g. guides) and colnames corresponding to samples.
- `sample.replicate.annotation`: a data.frame of annotations for each sample/replicate pair. Note that at least one column in `sample.replicate.annotation` must correspond to the colnames of `counts.matrix` (see Details) (default = NULL)
- `guide.annotation`: a data.frame of annotations for each guide. Note that at least one column in `guide.annotation` must correspond to the rownames of `counts.matrix` (default = NULL)
- `samplesAreColumns`: a logical indicating if samples are on the columns or rows of `counts.matrix`. (default = TRUE)
- `sample.column.name`: a character or integer indicating which column of `sample.replicate.annotation` describes the samples.
- `gene.column.names`: a character or integer vector of length(2) indicating which columns of `guide.annotation` describe the genes being targeted.
- `ETP.column`: a character or integer vector indicating which column(s) of `counts.matrix` contain the early time-point(s) of the screen (i.e. pDNA, early sequencing, etc.). Defaults to the first column.
- `LTP.column`: a character or integer vector indicating which column(s) is the later time-point of the screen (i.e. day21, post-treatment, etc.). Defaults to `(1:ncol(counts.matrix))[-ETP.column]`, or all other columns except for those specified by `ETP.column`.
- `verbose`: Verbosity (default FALSE)

Details

This function initializes a `gemini.input` object from a counts matrix. There are a few key assumptions made in the input format.
The counts matrix is regular.

The counts matrix structure is in accordance with the `samplesAreColumns` parameter.

The first column of `sample.replicate.annotation` matches with the existing dimension names of the counts matrix.

The first column of `guide.annotations` matches with the existing dimension names of the counts matrix.

`sample.column.name` must specify a column in `sample.replicate.annotation` (either by name or index) that describes unique samples.

`gene.column.names` must specify two columns in `sample.replicate.annotation` (either by name or index) that describe genes.

**Value**

a `gemini.input` object

**Examples**

```r
data("counts", package = "gemini")
data("sample.replicate.annotation", package = "gemini")
data("guide.annotation", package = "gemini")
Input <- gemini_create_input(
  counts.matrix = counts,
  sample.replicate.annotation = sample.replicate.annotation,
  guide.annotation = guide.annotation,
  sample.column.name = "samplename",
  gene.column.names = c("U6.gene", "H1.gene")
)
```

**Description**

Estimate the posterior using a variational inference technique. Inference is performed through an iterative process until convergence.

**Usage**

```r
gemini_inference(  
  Model,  
  n_iterations = 20,  
  mean_x = 1,  
  sd_x = 1,  
  mean_xx = 1,  
  sd_xx = 1,  
  mean_y = 0,  
)```
sd_y = 10,
mean_s = 0,
sd_s = 10,
threshold = 0.001,
cores = 1,
force_results = FALSE,
verbose = FALSE,
save_iterations = FALSE
)

Arguments

Model: an object of class gemini.model
n_iterations: a numeric indicating the maximum number of iterations (default=20).
mean_x: a numeric indicating prior mean of x (default=1).
sd_x: a numeric indicating prior sd of x (default=1).
mean_xx: a numeric indicating prior mean of xx (default=1).
sd_xx: a numeric indicating prior sd of xx (default=1).
mean_y: a numeric indicating prior mean of y (default=0).
sd_y: a numeric indicating prior sd of y (default=10).
mean_s: a numeric indicating prior mean of s (default=0).
sd_s: a numeric indicating prior sd of s (default=10).
threshold: a numeric indicating the threshold of change in MAE at which to stop the iterative process (default=0.001).
cores: a numeric indicating the number of cores to use. See details in gemini_parallel. (default=1)
force_results: a logical indicating if the CAVI algorithm should be halted if non-convergence is detected. (default=FALSE)
verbose: default FALSE
save_iterations: for especially large libraries that require long computations, saves the latest iteration of each update. default FALSE

Details

GEMINI uses the following parameters, which are described in Zamanighomi et al. and translated here for clarity:

- y: individual gene effect
- s: combination effect
- x: screen variation corresponding to individual guides
- xx: screen variation corresponding to paired guides

Default parameters may need to be changed if convergence is not achieved. See README for more details.
Value

a gemini.model object with estimated posteriors

Examples

data("Model", package = "gemini")
Model %<>% gemini_inference(verbose = FALSE, n_iterations = 1) # iterations set to 1 for testing

Description

Creates a gemini.model object given Input data and initialization parameters.

Usage

gemini_initialize(
  Input,
  guide.pair.annot = "guide.pair.annot",
  replicate.map = "replicate.map",
  sample.column.name = "samplename",
  LFC.name = "LFC",
  nc_gene,
  CONSTANT = 32,
  concordance = 1,
  prior_shape = 0.5,
  pattern_join = ";",
  pattern_split = ";",
  window = NULL,
  monotonize = FALSE,
  verbose = FALSE,
  cores = 1,
  save = NULL
)

Arguments

Input an object of class gemini.input
guide.pair.annot the name of an object within Input containing guide to gene annotations (Default = "guide.pair.annot")
replicate.map the name of an object within Input containing replicate and sample mappings (Default = "replicate.map")
sample.column.name  
  a character or integer indicating which column of Input$replicate.map describes the samples.

LFC.name  
  a character indicating an object within Input to treat as LFC. By default, "LFC" is used, which is the output of \texttt{gemini_calculate_lfc}.

nc_gene  
  a character naming the gene to use as a negative control. See details for more.

CONSTANT  
  a numeric indicating a constant value that shifts counts to reduce outliers, see Details (default = 32).

concordance  
  a numeric value to initialize x (default = 1)

prior_shape  
  shape parameter of Gamma distribution used to model the variation in the data in Input (default = 0.5)

pattern_join  
  a character to join the gene combinations found in guide.pair.annot. Default ";"

pattern_split  
  a character to split the guide combinations found in guide.pair.annot. Default ";"

window  
  a numeric if window smoothing should be done on initialized tau values, otherwise NULL (default) for no window smoothing

monotonize  
  a logical specifying whether the variance should be monotonically increasing. (default FALSE)

verbose  
  default FALSE

cores  
  numeric indicating the number of cores to use. See details in \texttt{gemini_parallelization}.

save  
  a character (file path) or logical indicating whether the initialized model should be saved.

\section*{Details}

\texttt{guide.pair.annot} is created with the following format:

\begin{itemize}
  \item 1st column contains guide pairs, joined by \texttt{pattern_split}.
  \item 2nd column contains the name of gene mapping to the first guide in the 1st column.
  \item 3rd column contains the name of gene mapping to the second guide in the 1st column.
\end{itemize}

Additional columns may be appended to \texttt{guide.pair.annot}, but the first three columns must be defined as above.

\textbf{Use of negative control} As described in Zamanighomi et al., it is highly recommended to specify a negative control. In the event that no negative control is specified, GEMINI will use the median LFC of all guide pairs targeting a gene to initialize that gene’s effect. While this may be reasonable in large all-by-all screens, it is not recommended in smaller screens or some-by-some screens. As a result, when possible, be sure to specify a negative control.

\section*{Value}

a Model object of class \texttt{gemini.model}
Examples

data("Input", package = "gemini")
Model <- gemini_initailize(Input, nc gene = "CD81")

Description

Notes about parallelization in combinatorial CRISPR analysis

Implementation

To improve efficiency and scalability, GEMINI employs parallelization, enabling a rapid initialization and update routine. Parallelization was implemented using the R pbmclapply package, and specifically through the pbmclapply function. As pbmclapply (and it’s parent function, mclapply) relies on forking, parallelization is currently limited to Unix-like (Linux flavors and macOS) machines, but may be extended to other OS in later versions through socket parallelization. In the event that GEMINI is used on a Windows machine, all computations are performed in serial.

Parallelized processes

GEMINI enables parallelization of both initialization and inference. For initialization, parallelization is used to quickly hash the data. For the inference procedure, parallelization is used to speed up the CAVI approach by performing updates independently across cores.

Caveats

To note, there is usually a trade-off in terms of number of cores and shared resources/memory transfer. See inst/figs/gemini-benchmarking.png for a visual depiction.

Also, while most functions in GEMINI have been parallelized, initialization of tau, update of x (group 3), and updates of y are performed in serial. As such, especially in large libraries, tau initialization and x (group 3) updates may take some time. Updating y is usually fast, as the number of genes tends to be the smallest in parameter space. However, these functions may be parallelized in the future as well.

Active Development

Noticed that in some cases, after using multicore processing through pbmclapply, warnings have been produced: "In selectChildren(pids[!fin], -1) : cannot wait for child ... as it does not exist" “In parallel::mcollect(...) : 1 parallel job did not deliver a result” R.version=3.6.0 These warnings, although they appear menacing, are in fact harmless. See: http://r.789695.n4.nabble.com/Strange-error-messages-from-parallel-mcparallel-family-under-3-6-0-td4756875.html#a4756939
**gemini_plot_mae**

*MAE plot*

**Description**

Plots the average MAE of gemini model at each iteration step.

**Usage**

`gemini_plot_mae(Model)`

**Arguments**

- **Model**: a gemini.model object

**Value**

a ggplot2 object

**Examples**

```r
data("Model", package = "gemini")
gemini_plot_mae(Model)
```

---

**gemini_prepare_input**

*Prepare input before Model creation*

**Description**

This is an internal function to GEMINI, allowing for data cleanup and preprocessing before a Model object is created. This removes any gene pairs targeting the same gene twice, and removes any empty samples/replicates.

**Usage**

`gemini_prepare_input(Input, gene.columns, sample.col.name = "samplename")`

**Arguments**

- **Input**: An object of class gemini.input
- **gene.columns**: a character vector of length(2)
- **sample.col.name**: a character indicating the name of the sample column (default = "samplename")
Value

A (prepared) `gemini.input` object

Examples

data("Input", package = "gemini")
Input %<>% gemini_prepare_input(gene.columns = c("U6.gene", "H1.gene"))

gemini_score

Scoring Combination Effect

Description

Score genetic interactions from a `gemini.model` and produce a `gemini.score` object, and generate p-values and FDRs if negative control pairs (`nc_pairs`) are available.

Usage

gemini_score(
  Model,
  pc_gene = NA,
  pc_threshold = NULL,
  pc_weight = 0.5,
  nc_pairs = NA
)

Arguments

- **Model**: an object of class `gemini.model`
- **pc_gene**: a character vector of any length naming genes to use as positive controls
- **pc_threshold**: a numeric value to indicate the LFC corresponding to a positive control, if no `pc_gene` is specified.
- **pc_weight**: a weighting applied to the positive control (`pc_gene`) to filter genes whose individual phenotype is more lethal than `pc_weight * y(pc_gene)`.
- **nc_pairs**: a set of non-interacting gene pairs to define statistical significance.

Value

An object of class `gemini.score` containing score values for strong interactions and sensitive lethality and recovery, and if `nc_pairs` is specified, statistical significance for each scoring type.

Examples

data("Model", package = "gemini")
Score <- gemini_score(Model, pc_gene = "EEF2")
guide.annotation

**Description**

Guide and gene annotations for the Big Papi SynLet dataset. The Big Papi dataset was published in Najm et al. 2018 (doi: 10.1038/nbt.4048). The guide and gene annotations were acquired from Supplementary Table 2.

**Usage**

guide.annotation

**Format**

An object of class `data.frame` with 9216 rows and 7 columns.

**See Also**

https://www.nature.com/articles/nbt.4048

initialize_s

**Description**

Initialize s values using initialized y values and data from Input

**Usage**

`initialize_s(Model, cores = 1, verbose = FALSE)`

**Arguments**

- **Model**: an object of class `gemini.model`
- **cores**: a numeric indicating the number of cores to use. See `gemini_parallelization` default 1.
- **verbose**: default FALSE

**Value**

A Model object of class `gemini.model` including new slots for `s` values

**Examples**

```r
data("Model", package = "gemini")
Model <- initialize_s(Model)
```
**initialize_tau**

Initialize all tau values based on the observed replicate variance.

**Usage**

```r
initialize_tau(
  Model,
  CONSTANT = 32,
  prior_shape = 0.5,
  window = NULL,
  monotonize = FALSE,
  verbose = FALSE
)
```

**Arguments**

- **Model**: an object of class `gemini.model`
- **CONSTANT**: a numeric indicating a constant value that shifts counts to reduce outliers (default = 32).
- **prior_shape**: shape parameter of Gamma distribution used to model the variation in the data input. If single numeric value, then shape parameters for all samples are assumed equal. Otherwise, a named numeric vector of shape parameters the same length as the number of samples (excluding early time point).
- **window**: numeric if window smoothing should be done on initialized tau values, otherwise `NULL` (default) for no window smoothing.
- **monotonize**: logical specifying whether the variance should be monotonically increasing (default `FALSE`).
- **verbose**: default `FALSE`.

**Value**

a `Model` object of class `gemini.model` including new slots for alpha and beta values.

**Examples**

```r
data("Model", package = "gemini")
Model <- initialize_tau(Model, CONSTANT = 32, prior_shape = 0.5)
```
**initialize_x**

Description

Initialize all x values to the value of concordance

Usage

initialize_x(Model, concordance = 1, cores = 1, verbose = FALSE)

Arguments

- **Model**
  a Model object of class gemini.model
- **concordance**
  a numeric value to initialize x
- **cores**
  a numeric indicating the number of cores to use. See `gemini_parallelization` default 1.
- **verbose**
  default FALSE

Value

a Model object of class gemini.model including new slots for x values and internal-use hashes

Note

As there is much hashing involved in this function, this tends to be computationally intensive. As such, we have enabled parallelization of most hash steps, but this may still be rate-limited by the amount of memory consumed.

Examples

data("Model", package = "gemini")
Model %<>% initialize_x()

**initialize_y**

Description

Initialize all y values from guide pairs including a negative control.

Usage

initialize_y(Model, verbose = FALSE, cores = 1)
### Input

**Arguments**

- **Model**: an object of class `gemini.model`
- **verbose**: default FALSE
- **cores**: a numeric indicating the number of cores to use. See details in `gemini_parallelization`. (default=1)

**Value**

a `Model` object of class `gemini.model` including new slots for \(y\) values

**Examples**

```r
data("Model", package = "gemini")
Model %<>% initialize_y()
```

---

**Description**

A `gemini.input` object created from the Big Papi SynLet dataset. The Big Papi dataset was published in Najm et al. 2018 (doi: 10.1038/nbt.4048). The `Input` object here is created from the data in `counts`, `guide.annotation`, and `sample.replicate.annotation` using the `gemini_create_input` function.

**Usage**

`Input`

**Format**

An object of class `list` (inherits from `gemini.input`, `gemini.input`) of length 6.

**See Also**

https://www.nature.com/articles/nbt.4048
**Model**

*Model object from Big Papi*

**Description**

A `gemini.model` object created from the Big Papi SynLet dataset after `gemini_inference` was run. The Big Papi dataset was published in Najm et al. 2018 (doi: 10.1038/nbt.4048). The Model object here is created from the data in Input using the `gemini_initialize` function.

**Usage**

- `Model`

**Format**

An object of class `list` (inherits from `gemini.model`) of length 24.

**See Also**

- [https://www.nature.com/articles/nbt.4048](https://www.nature.com/articles/nbt.4048)

---

**sample.replicate.annotation**

*Big Papi sample and replicate annotations*

**Description**

Sample and replicate annotations for the Big Papi SynLet dataset. The Big Papi dataset was published in Najm et al. 2018 (doi: 10.1038/nbt.4048). The sample and replicate annotations were acquired from Supplementary Table 3.

**Usage**

- `sample.replicate.annotation`

**Format**

An object of class `data.frame` with 16 rows and 3 columns.

**See Also**

- [https://www.nature.com/articles/nbt.4048](https://www.nature.com/articles/nbt.4048)
Sgene2Pguides_hash  

**Gene hashing**

**Description**
Gene hashing

**Usage**
Sgene2Pguides_hash(guide2gene, cores = 1)

**Arguments**
guide2gene  derived from Input object
cores  number of cores to use (default 1)

**Value**
Gene hash/list

**Examples**
```
## Not run:
#' data("Input", package = "gemini")
Sgene2Pguides_hash(Input$guide.pair.annot[1:10,])

## End(Not run)
```

Sguide2Pguides_hash  

**Guide hashing**

**Description**
Guide hashing

**Usage**
Sguide2Pguides_hash(guide2gene, split = ";", cores = 1)

**Arguments**
guide2gene  derived from Input object
split  character to split guides
cores  number of cores to use (default 1)
update_mae

Value

Guide hash/list

Examples

## Not run:
data("Input", package = "gemini")
Sguide2Pguides_hash(gemini::Input$guide.pair.annot[1:10,])

## End(Not run)

---

update_mae  update_mae

Description

Calculate mean absolute error across all guide combinations.

Usage

update_mae(Model, verbose = FALSE)

Arguments

Model  a Model object of class gemini.model
verbose default FALSE

Value

An object of class gemini.model

Examples

data("Model", package = "gemini")
Model %<>% update_mae()
**update_s_pb**

---

### Description

Update values of s using data from Input and current values of other parameters.

### Usage

```r
update_s_pb(Model, mean_s = 0, sd_s = 10, cores = 1, verbose = FALSE)
```

### Arguments

- **Model**: a Model object of class `gemini.model`
- **mean_s**: numeric indicating prior mean of s (default 0)
- **sd_s**: numeric indicating prior sd of s (default 10)
- **cores**: a numeric indicating the number of cores to use, see `gemini_parallelization`. default=1.
- **verbose**: default FALSE

### Value

An object of class `gemini.model`

### Examples

```r
data("Model", package = "gemini")
Model %<>% update_s_pb()
```

---

**update_tau_pb**

---

### Description

Update parameters of tau using data from Input and current values of other parameters.

### Usage

```r
update_tau_pb(Model, cores = 1, verbose = FALSE)
```
Arguments

- **Model**: a Model object of class `gemini.model`
- **cores**: a numeric indicating the number of cores to use. See `gemini_parallelization` for details. (default=1).
- **verbose**: default FALSE

Value

An object of class `gemini.model`

Examples

```r
data("Model", package = "gemini")
Model %<>% update_tau_pb()
```

---

**Description**

Update values of x using data from Input and current values of other parameters.

**Usage**

```r
update_x_pb(
  Model,
  mean_x = 1,
  sd_x = 1,
  mean_xx = 1,
  sd_xx = 1,
  cores = 1,
  verbose = FALSE
)
```

**Arguments**

- **Model**: a Model object of class `gemini.model`
- **mean_x**: a numeric indicating prior mean of x
- **sd_x**: a numeric indicating prior sd of x
- **mean_xx**: a numeric indicating prior mean of xx
- **sd_xx**: a numeric indicating prior sd of xx
- **cores**: a numeric indicating the number of cores to use. See `gemini_parallelization` for details. (default=1).
- **verbose**: default FALSE
update_y_pb

Value

An object of class gemini.model

Note

The structure of the screen may impede parallelization. Our ability to parallelize the updates is contingent upon the independence between guides in position 1 and 2. To account for potential dependence, we define three groups of guides: group 1 (guides only in position 1), group 2 (guides only in position 2), and group 3 (guides in both position 1 and position 2). Parallelization is possible for groups 1 and 2, but only serial updates are possible for group 3. As such, updates for this group will take longer.

Examples

data("Model", package = "gemini")
Model %<>% update_x_pb()

update_y_pb update_y_pb

Description

Update values of y using data from Input and current values of other parameters.

Usage

update_y_pb(Model, mean_y = 0, sd_y = 10, verbose = FALSE)

Arguments

Model a Model object of class gemini.model
mean_y numeric indicating prior mean of y
sd_y numeric indicating prior sd of y
verbose default FALSE

Value

An object of class gemini.model

Examples

data("Model", package = "gemini")
Model %<>% update_y_pb()
%<>%  

**Compound pipe**

**Description**

Compound pipe

**Arguments**

 lhs, rhs  
 An object modified in place, and a function to apply to it

**Examples**

```
a <- 1:5
a %<>% sum() # a is modified in place
```

%>%

**Pipe**

**Description**

Pipe

**Arguments**

 lhs, rhs  
 An object, and a function to apply to it

**Examples**

```
a <- 1:5
b <- a %>% sum()
```
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