

Package ‘dominoSignal’

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Title Cell Communication Analysis for Single Cell RNA Sequencing

Version 1.3.0

Description dominoSignal is a package developed to analyze cell signaling through ligand - receptor - transcription factor networks in scRNAseq data. It takes as input information transcriptional data, requiring counts, z-scored counts, and cluster labels, as well as information on transcription factor activation (such as from SCENIC) and a database of ligand and receptor pairings (such as from CellPhoneDB). This package creates an object storing ligand - receptor - transcription factor linkages by cluster and provides several methods for exploring, summarizing, and visualizing the analysis.

BugReports <https://github.com/FertigLab/dominoSignal/issues>

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<code>add_rl_column</code>	<i>Adds a column to the RL signaling data frame.</i>
----------------------------	--

Description

This function adds a column to the internal `rl` 'map' used to map all receptor and receptor complexes to all ligand and ligand complexes.

Usage

```
add_rl_column(map, map_ref, conv, new_name)
```

Arguments

<code>map</code>	RL signaling data frame.
<code>map_ref</code>	Name of column to match new data to
<code>conv</code>	Data frame matching current data in map to new data.
<code>new_name</code>	Name of new column to be created in RL map

Value

An updated RL signaling data frame

Examples

```
example(create_rl_map_cellphonedb, echo = FALSE)
lr_name <- data.frame("abbrev" = c("L", "R"), "full" = c("Ligand", "Receptor"))
rl_map_expanded <- add_rl_column(map = rl_map_tiny, map_ref = "type_A",
conv = lr_name, new_name = "type_A_full")
```

avg_exp_for_complexes *Get average expression for complexes*

Description

Get average expression for complexes

Usage

```
avg_exp_for_complexes(exp_mat, complexes_list)
```

Arguments

exp_mat A matrix(or dataframe) of genes x clusters, values are z-scores averaged over the clusters

complexes_list A list similar to dom@linkages\$complexes

Value

A list containing average expression for any complexes

build_domino *Calculate a signaling network for a domino object*

Description

This function calculates a signaling network. It requires a domino object preprocessed from create_domino and returns a domino object prepared for plotting with the various plotting functions in this package.

Usage

```
build_domino(
  dom,
  max_tf_per_clust = 5,
  min_tf_pval = 0.01,
  max_rec_per_tf = 5,
  rec_tf_cor_threshold = 0.15,
  min_rec_percentage = 0.1
)
```

Arguments

dom Domino object from [create_domino\(\)](#).

max_tf_per_clust Maximum number of transcription factors called active in a cluster.

min_tf_pval Minimum p-value from differential feature score test to call a transcription factor active in a cluster.

max_rec_per_tf Maximum number of receptors to link to each transcription factor.

`rec_tf_cor_threshold`

Minimum Spearman correlation used to consider a receptor linked with a transcription factor. Increasing this will decrease the number of receptors linked to each transcription factor.

`min_rec_percentage`

Minimum percentage of cells in cluster expressing a receptor for the receptor to be linked to transcription factors in that cluster.

Value

A domino object with a signaling network built

Examples

```
example(create_domino, echo = FALSE)
```

```
#a relaxed example
```

```
pbmc_dom_built_tiny <- build_domino(
  dom = pbmc_dom_tiny, min_tf_pval = .05, max_tf_per_clust = Inf,
  max_rec_per_tf = Inf, rec_tf_cor_threshold = .1, min_rec_percentage = 0.01
)
```

CellPhoneDB

CellPhoneDB subset

Description

A list of four subsets of CellPhoneDB data.

Usage

```
data("CellPhoneDB")
```

Format

A list of:

genes_tiny A subset of CellPhoneDB gene_input.csv

proteins_tiny A subset of CellPhoneDB protein_input.csv

complexes_tiny A subset of CellPhoneDB complex_input.csv

interactions_tiny A subset of CellPhoneDB interaction_input.csv

Source

<https://github.com/ventolab/cellphonedb-data/archive/refs/tags/v4.0.0.tar.gz>

check_arg	<i>Check input arguments</i>
-----------	------------------------------

Description

Accepts an object and rules to check against; stops if requirements are not met

Usage

```
check_arg(
  arg,
  allow_class = NULL,
  allow_len = NULL,
  allow_range = NULL,
  allow_values = NULL,
  need_vars = c(NULL),
  need_colnames = FALSE,
  need_rownames = FALSE,
  need_names = FALSE
)
```

Arguments

arg	the argument to check
allow_class	vector of allowed classes
allow_len	vector of allowed lengths
allow_range	range of minimum and maximum values i.e. c(1, 5)
allow_values	vector of allowed values
need_vars	vector of required variables
need_colnames	logical for whether colnames are required
need_rownames	logical for whether rownames are required
need_names	logical for whether names are required

Value

Logical indicating whether the argument meets the requirements

circos_ligand_receptor	<i>Plot expression of a receptor's ligands by other cell types as a chord plot</i>
------------------------	--

Description

Creates a chord plot of expression of ligands that can activate a specified receptor where chord widths correspond to mean ligand expression by the cluster.

Usage

```

circos_ligand_receptor(
  dom,
  receptor,
  ligand_expression_threshold = 0.01,
  cell_idents = NULL,
  cell_colors = NULL
)

```

Arguments

dom	Domino object that has undergone network building with build_domino()
receptor	Name of a receptor active in at least one cell type in the domino object
ligand_expression_threshold	Minimum mean expression value of a ligand by a cell type for a chord to be rendered between the cell type and the receptor
cell_idents	Vector of cell types from cluster assignments in the domino object to be included in the plot.
cell_colors	Named vector of color names or hex codes where names correspond to the plotted cell types and the color values

Value

Renders a circos plot to the active graphics device

Examples

```

example(build_domino, echo = FALSE)
#basic usage
circos_ligand_receptor(pbmcdom_built_tiny, receptor = "CXCR3")
#specify colors
cols = c("red", "orange", "green")
names(cols) = dom_clusters(pbmcdom_built_tiny)
circos_ligand_receptor(pbmcdom_built_tiny, receptor = "CXCR3", cell_colors = cols)

```

convert_genes

Use biomaRt to convert genes

Description

This function reads in a vector of genes and converts the genes to specified symbol type

Usage

```

convert_genes(
  genes,
  from = c("ENSMUSG", "ENSG", "MGI", "HGNC"),
  to = c("MGI", "HGNC"),
  host = "https://www.ensembl.org"
)

```

Arguments

genes	Vector of genes to convert.
from	Format of gene input (ENSMUSG, ENSG, MGI, or HGNC)
to	Format of gene output (MGI or HGNC)
host	Host to connect to. Defaults to https://www.ensembl.org following the useMart default, but can be changed to archived hosts if useMart fails to connect.

Value

A data frame with input genes as column 1 and converted genes as column 2

conv_py_bools	<i>Change cases of True/False syntax from Python to TRUE/FALSE R syntax</i>
---------------	---

Description

Change cases of True/False syntax from Python to TRUE/FALSE R syntax

Usage

```
conv_py_bools(obj)
```

Arguments

obj	object that will be converted
-----	-------------------------------

Value

The converted object

cor_heatmap	<i>Create a heatmap of correlation between receptors and transcription factors</i>
-------------	--

Description

Creates a heatmap of correlation values between receptors and transcription factors either with boolean threshold or with continuous values displayed

Usage

```
cor_heatmap(
  dom,
  bool = FALSE,
  bool_thresh = 0.15,
  title = TRUE,
  feats = NULL,
  recs = NULL,
  mark_connections = FALSE,
  ...
)
```

Arguments

dom	Domino object with network built (build_domino())
bool	Boolean indicating whether the heatmap should be continuous or boolean. If boolean then bool_thresh will be used to determine how to define activity as positive or negative.
bool_thresh	Numeric indicating the threshold separating 'on' or 'off' for feature activity if making a boolean heatmap.
title	Either a string to use as the title or a boolean describing whether to include a title. In order to pass the 'main' parameter to ComplexHeatmap::Heatmap() you must set title to FALSE.
feats	Either a vector of features to include in the heatmap or 'all' for all features. If left NULL then the features selected for the signaling network will be shown.
recs	Either a vector of receptors to include in the heatmap or 'all' for all receptors. If left NULL then the receptors selected in the signaling network connected to the features plotted will be shown.
mark_connections	Boolean indicating whether to add an 'x' in cells where there is a connected receptor or TF. Default FALSE.
...	Other parameters to pass to ComplexHeatmap::Heatmap() . Note that to use the 'main' parameter of ComplexHeatmap::Heatmap() you must set title = FALSE and to use 'annCol' or 'annColors' ann_cols must be FALSE.

Value

A heatmap rendered to the active graphics device

Examples

```
example(build_domino, echo = FALSE)
#basic usage
cor_heatmap(pbmc_dom_built_tiny, title = "PBMC R-TF Correlations")
#show correlations above a specific value
cor_heatmap(pbmc_dom_built_tiny, bool = TRUE, bool_thresh = 0.1)
#identify combinations that are connected
cor_heatmap(pbmc_dom_built_tiny, bool = FALSE, mark_connections = TRUE)
```

cor_scatter

Create a correlation plot between TF and receptor

Description

Create a correlation plot between transcription factor activation score and receptor expression

Usage

```
cor_scatter(dom, tf, rec, remove_rec_dropout = TRUE, ...)
```

Arguments

dom	Domino object with network built (build_domino())
tf	Target TF for plotting AUC score
rec	Target receptor for plotting expression
remove_rec_dropout	Whether to remove cells with zero expression for plot. This should match the same setting as in build_domino() .
...	Other parameters to pass to ggpubr::ggscatter() .

Value

A ggplot scatter plot rendered in the active graphics device

Examples

```
example(build_domino, echo = FALSE)
cor_scatter(pbmc_dom_built_tiny, "FLI1", "CXCR3")
```

count_linkage	<i>Count occurrences of linkages across multiple domino results from a linkage summary</i>
---------------	--

Description

Count occurrences of linkages across multiple domino results from a linkage summary

Usage

```
count_linkage(
  linkage_summary,
  cluster,
  group.by = NULL,
  linkage = "rec_lig",
  subject_names = NULL
)
```

Arguments

linkage_summary	a linkage_summary() object
cluster	the name of the cell cluster being compared across multiple domino results
group.by	the name of the column in <code>linkage_summary@subject_meta</code> by which to group subjects for counting. If NULL, only total counts of linkages for linkages in the cluster across all subjects is given.
linkage	a stored linkage from the domino object. Can compare any of 'tfs', 'rec', 'incoming_lig', 'tfs_rec', or 'rec_lig'
subject_names	a vector of subject_names from the linkage_summary to be compared. If NULL, all subject_names in the linkage summary are included in counting.

Value

A data frame with columns for the unique linkage features and the counts of how many times the linkage occurred across the compared domino results. If `group.by` is used, counts of the linkages are also provided as columns named by the unique values of the `group.by` variable.

Examples

```
count_linkage(
  linkage_summary = mock_linkage_summary(), cluster = "C1",
  group.by = "group", linkage = "rec")
```

create_domino	Create a domino object and prepare it for network construction
---------------	--

Description

This function reads in a receptor ligand signaling database, cell level features of some kind (ie. output from pySCENIC), z-scored single cell data, and cluster id for single cell data, calculates a correlation matrix between receptors and other features (this is transcription factor module scores if using pySCENIC), and finds features enriched by cluster. It will return a domino object prepared for `build_domino()`, which will calculate a signaling network.

Usage

```
create_domino(
  rl_map,
  features,
  counts = NULL,
  z_scores = NULL,
  clusters = NULL,
  use_clusters = TRUE,
  tf_targets = NULL,
  verbose = TRUE,
  use_complexes = TRUE,
  rec_min_thresh = 0.025,
  remove_rec_dropout = TRUE,
  tf_selection_method = "clusters",
  tf_variance_quantile = 0.5
)
```

Arguments

rl_map	Data frame where each row describes a receptor-ligand interaction with required columns <code>gene_A</code> & <code>gene_B</code> including the gene names for the receptor and ligand and <code>type_A</code> & <code>type_B</code> annotating if genes A and B are a ligand (L) or receptor (R)
features	Either a path to a csv containing cell level features of interest (ie. the auc matrix from pySCENIC) or named matrix with cells as columns and features as rows.
counts	Counts matrix for the data. This is only used to threshold receptors on dropout.

<code>z_scores</code>	Matrix containing z-scored expression data for all cells with cells as columns and features as rows.
<code>clusters</code>	Named factor containing cell cluster with names as cells.
<code>use_clusters</code>	Boolean indicating whether to use clusters.
<code>tf_targets</code>	Optional. A list where names are transcription factors and the stored values are character vectors of genes in the transcription factor's regulon.
<code>verbose</code>	Boolean indicating whether or not to print progress during computation.
<code>use_complexes</code>	Boolean indicating whether you wish to use receptor/ligand complexes in the receptor ligand signaling database. If FALSE, receptor/ligand pairs where either functions as a protein complex will not be considered when constructing the signaling network.
<code>rec_min_thresh</code>	Minimum expression level of receptors by cell. Default is 0.025 or 2.5 percent of all cells in the data set. This is important when calculating correlation to connect receptors to transcription activation. If this threshold is too low then correlation calculations will proceed with very few cells with non-zero expression.
<code>remove_rec_dropout</code>	Whether to remove receptors with 0 expression counts when calculating correlations. This can reduce false positive correlation calculations when receptors have high dropout rates.
<code>tf_selection_method</code>	Selection of which method to target transcription factors. If 'clusters' then differential expression for clusters will be calculated. If 'variable' then the most variable transcription factors will be selected. If 'all' then all transcription factors in the feature matrix will be used. Default is 'clusters'. Note that if you wish to use clusters for intercellular signaling downstream to MUST choose clusters.
<code>tf_variance_quantile</code>	What proportion of variable features to take if using variance to threshold features. Default is 0.5. Higher numbers will keep more features. Ignored if <code>tf_selection_method</code> is not 'variable'

Value

A domino object

Examples

```
example(create_rl_map_cellphonedb, echo = FALSE)
example(create_regulon_list_scenic, echo = FALSE)
data(SCENIC)
data(PBMC)

pbmc_dom_tiny <- create_domino(
  rl_map = rl_map_tiny, features = SCENIC$auc_tiny,
  counts = PBMC$RNA_count_tiny, z_scores = PBMC$RNA_zscore_tiny,
  clusters = PBMC$clusters_tiny, tf_targets = regulon_list_tiny,
  use_clusters = TRUE, use_complexes = TRUE, remove_rec_dropout = FALSE,
  verbose = FALSE
)

pbmc_dom_tiny_no_clusters <- create_domino(
  rl_map = rl_map_tiny, features = SCENIC$auc_tiny,
  counts = PBMC$RNA_count_tiny, z_scores = PBMC$RNA_zscore_tiny,
```

```
clusters = PBMC$clusters_tiny, tf_targets = regulon_list_tiny,
use_clusters = FALSE, use_complexes = FALSE,
rec_min_thresh = 0.1, remove_rec_dropout = TRUE,
tf_selection_method = "all",
verbose = FALSE
)
```

```
create_regulon_list_scenic
```

Create a list of genes in regulons inferred by SCENIC

Description

Generates a list of transcription factors and the genes targeted by the transcription factor as part of their regulon inferred by pySCENIC

Usage

```
create_regulon_list_scenic(regulons)
```

Arguments

regulons Data frame or file path to the table of the output of the ctx function from pySCENIC

Value

A list where names are transcription factors and the stored values are character vectors of genes in the inferred regulons

Examples

```
data(SCENIC)
regulon_list_tiny <- create_regulon_list_scenic(regulons = SCENIC$regulons_tiny)
```

```
create_rl_map_cellphonedb
```

Create a receptor - ligand map from a CellPhoneDB signaling database

Description

Generates a data frame of ligand-receptor interactions from a CellPhoneDB database annotating the genes encoding the interacting ligands and receptors to be queried in transcriptomic data.

Usage

```
create_rl_map_cellphonedb(
  genes,
  proteins,
  interactions,
  complexes = NULL,
  database_name = "CellPhoneDB",
  gene_conv = NULL,
  gene_conv_host = "https://www.ensembl.org",
  alternate_convert = FALSE,
  alternate_convert_table = NULL
)
```

Arguments

<code>genes</code>	data frame or file path to table of gene names in uniprot, hgnc_symbol, or ensembl format in CellPhoneDB database format
<code>proteins</code>	data frame or file path to table of protein features in CellPhoneDB format
<code>interactions</code>	data frame or file path to table of protein-protein interactions in CellPhoneDB format
<code>complexes</code>	optional: data frame or file path to table of protein complexes in CellPhoneDB format
<code>database_name</code>	name of the database being used, stored in output
<code>gene_conv</code>	a tuple of (from, to) or (source, target) if gene conversion to orthologs is desired; options are ENSMUSG, ENSG, MGI, or HGNC
<code>gene_conv_host</code>	host for conversion; default ensembl, could also use mirrors if desired
<code>alternate_convert</code>	boolean if you would like to use a non-ensembl method of conversion (must supply table; not recommended, use only if ensembl is down)
<code>alternate_convert_table</code>	supplied table for non-ensembl method of conversion

Value

Data frame where each row describes a possible receptor-ligand interaction

Examples

```
data(CellPhoneDB)
rl_map_tiny <- create_rl_map_cellphonedb(genes = CellPhoneDB$genes_tiny,
  proteins = CellPhoneDB$proteins_tiny,
  interactions = CellPhoneDB$interactions_tiny,
  complexes = CellPhoneDB$complexes_tiny)
```

domino-class

The domino class

Description

The domino class contains all information necessary to calculate receptor-ligand signaling. It contains z-scored expression, cell cluster labels, feature values, and a referenced receptor-ligand database formatted as a receptor-ligand map. Calculated intermediate values are also stored.

Value

An instance of class domino

Slots

db_info List of data sets from ligand - receptor database
 counts Raw count gene expression data
 z_scores Matrix of z-scored expression data with cells as columns
 clusters Named factor with cluster identity of each cell
 features Matrix of features (TFs) to correlate receptor - ligand expression with. Cells are columns and features are rows.
 cor Correlation matrix of receptor expression to features.
 linkages List of lists containing info linking cluster->tf->rec->lig
 clust_de Data frame containing differential expression results for features by cluster.
 misc List of miscellaneous info pertaining to run parameters etc.
 cl_signaling_matrices Incoming signaling matrix for each cluster
 signaling Signaling matrix between all clusters.

dom_clusters

Access clusters

Description

A function to pull cluster information from a domino object

Usage

```
dom_clusters(dom, labels = FALSE)
```

Arguments

dom	a domino object that has been created with <code>create_domino()</code>
labels	a boolean for whether to return the cluster labels for each cell or the clusters used for inferring communication

Value

A vector containing either the names of the clusters used OR factors of the cluster label for each individual cell

Examples

```
example(build_domino, echo = FALSE)
cluster_names <- dom_clusters(pbmc_dom_built_tiny)
cell_cluster_label <- dom_clusters(pbmc_dom_built_tiny, labels = TRUE)
```

dom_correlations	<i>Access correlations</i>
------------------	----------------------------

Description

A function to pull receptor-transcription factor correlations from a domino object

Usage

```
dom_correlations(dom, type = "rl")
```

Arguments

dom	a domino object that has been created with create_domino()
type	either "rl" or "complex", to select between the receptor-ligand or complex correlation matrix

Value

A matrix containing the correlation values for each receptor (row) by transcription factor (column)

Examples

```
example(build_domino, echo = FALSE)
cor_matrix <- dom_correlations(pbmc_dom_built_tiny, "rl")
```

dom_counts	<i>Access counts</i>
------------	----------------------

Description

A function to pull gene expression from a domino object

Usage

```
dom_counts(dom)
```

Arguments

dom a domino object that has been created with `create_domino()`

Value

A matrix containing the gene expression values for each gene (row) by cell (column)

Examples

```
example(build_domino, echo = FALSE)
counts <- dom_counts(pbmc_dom_built_tiny)
```

dom_database	<i>Access database</i>
--------------	------------------------

Description

A function to pull database information from a domino object

Usage

```
dom_database(dom, name_only = TRUE)
```

Arguments

dom a domino object that has been created

name_only a boolean for whether to return only the name of the database used or the entire database that is stored. Default TRUE.

Value

A vector of unique databases used in building the domino object OR a data frame that includes the database information used in the domino object creation

Examples

```
example(build_domino, echo = FALSE)
database_name <- dom_database(pbmc_dom_built_tiny)
full_database <- dom_database(pbmc_dom_built_tiny, name_only = FALSE)
```

dom_de	<i>Access differential expression</i>
--------	---------------------------------------

Description

A function to pull differential expression p-values from a domino object

Usage

```
dom_de(dom)
```

Arguments

dom a domino object that has been created with `create_domino()`

Value

A matrix containing the p-values for differential expression of transcription factors (rows) in each cluster (columns)

Examples

```
example(build_domino, echo = FALSE)
de_mat <- dom_de(pbmcdom_built_tiny)
```

dom_info	<i>Access build information</i>
----------	---------------------------------

Description

A function to pull the parameters used when running `build_domino()` from a domino object

Usage

```
dom_info(dom)
```

Arguments

dom a domino object that has been created with `create_domino()`

Value

A list containing booleans for whether the object has been created and built and a list of the build parameters that were used in `build_domino()` to infer the signaling network

Examples

```
example(build_domino, echo = FALSE)
build_details <- dom_info(pbmcdom_built_tiny)
```

dom_linkages	<i>Access linkages</i>
--------------	------------------------

Description

A function to pull linkages from a domino object

Usage

```
dom_linkages(
  dom,
  link_type = c("complexes", "receptor-ligand", "tf-target", "tf-receptor", "receptor",
    "incoming-ligand"),
  by_cluster = FALSE
)
```

Arguments

dom	a domino object that has been created with <code>create_domino()</code>
link_type	one value (out of "complexes", "receptor-ligand", "tf-target", "tf-receptor", "receptor", "incoming-ligand") used to select the desired type of linkage
by_cluster	a boolean to indicate whether the linkages should be returned overall or by cluster

Value

A list containing linkages between some combination of receptors, ligands, transcription factors, and clusters

Examples

```
example(build_domino, echo = FALSE)
complexes <- dom_linkages(pbmcdom_built_tiny, "complexes")
tf_rec_by_cluster <- dom_linkages(pbmcdom_built_tiny, "tf-receptor", TRUE)
```

dom_network_items	<i>Access all features, receptors, or ligands present in a signaling network.</i>
-------------------	---

Description

This function collates all of the features, receptors, or ligands found in a signaling network anywhere in a list of clusters. This can be useful for comparing signaling networks across two separate conditions. In order to run this `build_domino()` must be run on the object previously.

Usage

```
dom_network_items(dom, clusters = NULL, return = NULL)
```

Arguments

dom	a domino object containing a signaling network (i.e. <code>build_domino()</code> was run)
clusters	vector indicating clusters to collate network items from. If left as NULL then all clusters will be included.
return	string indicating whether to collate "features", "receptors", or "ligands". If "all" then a list of all three will be returned.

Value

A vector containing all features, receptors, or ligands in the data set or a list containing all three.

Examples

```
example(build_domino, echo = FALSE)
monocyte_receptors <- dom_network_items(pbmcdom_built_tiny, "CD14_monocyte", "receptors")
all_tfs <- dom_network_items(pbmcdom_built_tiny, return = "features")
```

dom_signaling

Access signaling

Description

A function to pull signaling matrices from a domino object

Usage

```
dom_signaling(dom, cluster = NULL)
```

Arguments

dom	a domino object that has been created with <code>create_domino()</code>
cluster	either NULL to indicate global signaling or a specific cluster for which a signaling matrix is desired

Value

A data frame containing the signaling score through each ligand (row) by each cluster (column) OR a data frame containing the global summed signaling scores between receptors (rows) and ligands (columns) of each cluster

Examples

```
example(build_domino, echo = FALSE)
monocyte_signaling <- dom_signaling(pbmcdom_built_tiny, cluster = "CD14_monocyte")
```

dom_tf_activation	<i>Access transcription factor activation</i>
-------------------	---

Description

A function to pull transcription factor activation scores from a domino object

Usage

```
dom_tf_activation(dom)
```

Arguments

dom a domino object that has been created with `create_domino()`

Value

A matrix containing the transcription factor activation scores for each TF (row) by cell (column)

Examples

```
example(build_domino, echo = FALSE)
tf_activation <- dom_tf_activation(pbmcdom_built_tiny)
```

dom_zscores	<i>Access z-scores</i>
-------------	------------------------

Description

A function to pull z-scored expression from a domino object

Usage

```
dom_zscores(dom)
```

Arguments

dom a domino object that has been created with `create_domino()`

Value

A matrix containing the z-scored gene expression values for each gene (row) by cell (column)

Examples

```
example(build_domino, echo = FALSE)
zscores <- dom_zscores(pbmcdom_built_tiny)
```

do_norm	<i>Normalize a matrix to its max value by row or column</i>
---------	---

Description

Normalizes a matrix to its max value by row or column

Usage

```
do_norm(mat, dir)
```

Arguments

mat	Matrix to be normalized
dir	Direction to normalize the matrix (either "row" for row or "col" for column)

Value

A normalized matrix in the direction specified.

feat_heatmap	<i>Create a heatmap of features organized by cluster</i>
--------------	--

Description

Creates a heatmap of transcription factor activation scores by cells grouped by cluster.

Usage

```
feat_heatmap(
  dom,
  feats = NULL,
  bool = FALSE,
  bool_thresh = 0.2,
  title = TRUE,
  norm = FALSE,
  cols = NULL,
  ann_cols = TRUE,
  min_thresh = NULL,
  max_thresh = NULL,
  ...
)
```

Arguments

dom	Domino object with network built (build_domino())
feats	Either a vector of features to include in the heatmap or 'all' for all features. If left NULL then the features selected for the signaling network will be shown.
bool	Boolean indicating whether the heatmap should be continuous or boolean. If boolean then bool_thresh will be used to determine how to define activity as positive or negative.
bool_thresh	Numeric indicating the threshold separating 'on' or 'off' for feature activity if making a boolean heatmap.
title	Either a string to use as the title or a boolean describing whether to include a title. In order to pass the 'main' parameter to ComplexHeatmap::Heatmap() you must set title to FALSE.
norm	Boolean indicating whether or not to normalize the transcription factors to their max value.
cols	Named vector of colors to annotate cells by cluster color. Values are taken as colors and names as cluster. If left as NULL then default ggplot colors will be generated.
ann_cols	Boolean indicating whether to include cell cluster as a column annotation. Colors can be defined with cols. If FALSE then custom annotations can be passed to ComplexHeatmap::Heatmap() .
min_thresh	Minimum threshold for color scaling if not a boolean heatmap
max_thresh	Maximum threshold for color scaling if not a boolean heatmap
...	Other parameters to pass to ComplexHeatmap::Heatmap() . Note that to use the 'main' parameter of ComplexHeatmap::Heatmap() you must set title = FALSE and to use 'annCol' or 'annColors' ann_cols must be FALSE.

Value

A heatmap rendered to the active graphics device

Examples

```
#basic usage
example(build_domino, echo = FALSE)
feat_heatmap(pbmc_dom_built_tiny)
#using thresholds
feat_heatmap(
  pbmc_dom_built_tiny, min_thresh = 0.1,
  max_thresh = 0.6, norm = TRUE, bool = FALSE)
```

gene_network

Create a gene association network

Description

Create a gene association network for genes from a given cluster. The selected cluster acts as the receptor for the gene association network, so only ligands, receptors, and features associated with the receptor cluster will be included in the plot.

Usage

```
gene_network(
  dom,
  clust = NULL,
  OutgoingSignalingClust = NULL,
  class_cols = c(lig = "#FF685F", rec = "#47a7ff", feat = "#39C740"),
  cols = NULL,
  lig_scale = 1,
  layout = "grid",
  ...
)
```

Arguments

dom	Domino object with network built (build_domino())
clust	Receptor cluster to create the gene association network for. A vector of clusters may be provided.
OutgoingSignalingClust	Vector of clusters to plot the outgoing signaling from
class_cols	Named vector of colors used to color classes of vertices. Values must be colors and names must be classes ('rec', 'lig', and 'feat' for receptors, ligands, and features.).
cols	Named vector of colors for individual genes. Genes not included in this vector will be colored according to class_cols.
lig_scale	FALSE or a numeric value to scale the size of ligand vertices based on z-scored expression in the data set.
layout	Type of layout to use. Options are 'grid', 'random', 'sphere', 'circle', 'fr' for Fruchterman-Reingold force directed layout, and 'kk' for Kamada Kawai for directed layout.
...	Other parameters to pass to plot() with an igraph object. See igraph manual for options.

Value

An igraph plot rendered to the active graphics device

Examples

```
#basic usage
example(build_domino, echo = FALSE)
gene_network(
  pbmc_dom_built_tiny, clust = "CD8_T_cell",
  OutgoingSignalingClust = "CD14_monocyte")
```

ggplot_col_gen	<i>Generate ggplot colors</i>
----------------	-------------------------------

Description

Accepts a number of colors to generate and generates a ggplot color spectrum.

Usage

```
ggplot_col_gen(n)
```

Arguments

n	Number of colors to generate
---	------------------------------

Value

A vector of colors according to ggplot color generation.

incoming_signaling_heatmap	<i>Create a cluster incoming signaling heatmap</i>
----------------------------	--

Description

Creates a heatmap of a cluster incoming signaling matrix. Each cluster has a list of ligands capable of activating its enriched transcription factors. The function creates a heatmap of cluster average expression for all of those ligands. A list of all cluster incoming signaling matrices can be found in the `cl_signaling_matrices` slot of a domino option as an alternative to this plotting function.

Usage

```
incoming_signaling_heatmap(
  dom,
  rec_clust,
  clusts = NULL,
  min_thresh = -Inf,
  max_thresh = Inf,
  scale = "none",
  normalize = "none",
  title = TRUE,
  ...
)
```

Arguments

dom	Domino object with network built (build_domino())
rec_clust	Which cluster to select as the receptor. Must match naming of clusters in the domino object.
clusts	Vector of clusters to be included. If NULL then all clusters are used.
min_thresh	Minimum signaling threshold for plotting. Defaults to -Inf for no threshold.
max_thresh	Maximum signaling threshold for plotting. Defaults to Inf for no threshold.
scale	How to scale the values (after thresholding). Options are 'none', 'sqrt' for square root, or 'log' for log10.
normalize	Options to normalize the matrix. Accepted inputs are 'none' for no normalization, 'rec_norm' to normalize to the maximum value with each receptor cluster, or 'lig_norm' to normalize to the maximum value within each ligand cluster
title	Either a string to use as the title or a boolean describing whether to include a title. In order to pass the 'main' parameter to ComplexHeatmap::Heatmap() you must set title to FALSE.
...	Other parameters to pass to ComplexHeatmap::Heatmap() . Note that to use the 'column_title' parameter of ComplexHeatmap::Heatmap() you must set title = FALSE

Value

a Heatmap rendered to the active graphics device

Examples

```
example(build_domino, echo = FALSE)
#incoming signaling of the CD8 T cells
incoming_signaling_heatmap(pbmc_dom_built_tiny, "CD8_T_cell")
```

 lc

Pulls all items from a list pooled into a single vector

Description

Helper function to convert from a nested series of lists to a single vector.

Usage

```
lc(list, list_names)
```

Arguments

list	List to pull items from
list_names	Names of items in list to pool

Value

A vector containing all items in the list by list_names

linkage_summary-class *The domino linkage summary class*

Description

The linkage summary class contains linkages established in multiple domino objects through gene regulatory network inference and reference to receptor- ligand data bases. A data frame summarizing meta features that describe the domino objects compared in the linkage summary facilitates comparisons of established linkages and differential signaling interactions across categorical sample covariates.

Value

an instance of class linkage_summary

Slots

subject_names unique names for each domino result included in the summary

subject_meta data.frame with each row describing one subject and columns describing features of the subjects by which to draw comparisons of signaling networks

subject_linkages nested list of linkages inferred for each subject. Lists are stored in a heirarchical structure of subject-cluster-linkage where linkages include transcription factors (tfs), linkages between transcription factors and receptors (tfs_rec), active receptors (rec), possible receptor-ligand interactions (rec_lig), and incoming ligands (incoming_lig)

mean_exp_by_cluster *Get average expression for a set of genes over cluster(s)*

Description

Get average expression for a set of genes over cluster(s)

Usage

```
mean_exp_by_cluster(dom, clusts, genes)
```

Arguments

dom	A domino object
clusts	Cluster(s) for which we want to get average expression
genes	The genes for which we want to get average expression

Value

A dataframe of genes x clusters, values are z-scores averaged over the clusters

```
mean_ligand_expression
```

Calculate mean ligand expression as a data frame for plotting in circos plot

Description

Creates a data frame of mean ligand expression for use in plotting a circos plot of ligand expression and saving tables of mean expression. us

Usage

```
mean_ligand_expression(x, ligands, cell_ident, cell_barcodes, destination)
```

Arguments

x	Gene by cell expression matrix
ligands	Character vector of ligand genes to be quantified
cell_ident	Vector of cell type (identity) names for which to calculate mean ligand gene expression
cell_barcodes	Vector of cell barcodes (colnames of x) belonging to cell_ident to calculate mean expression across
destination	Name of the receptor with which each ligand interacts

Value

A data frame of ligand expression targeting the specified receptor

Examples

```
example(build_domino, echo = FALSE)
counts <- dom_counts(pbmcdom_built_tiny)
mean_exp <- mean_ligand_expression(counts,
  ligands = c("PTPRC", "FASLG"), cell_ident = "CD14_monocyte",
  cell_barcodes = colnames(counts), destination = "FAS")
```

```
mock_linkage_summary
```

Create a mock linkage summary object

Description

Create a mock linkage summary object

Usage

```
mock_linkage_summary()
```

Value

obj a linkage summary object

```
obtain_circos_expression
```

Obtain Circos Expression

Description

Pull expression data from a domino object and format for plotting as a receptor-oriented circos plot.

Usage

```
obtain_circos_expression(  
  dom,  
  receptor,  
  ligands,  
  ligand_expression_threshold = 0.01,  
  cell_idents = NULL  
)
```

Arguments

dom	Domino object that has undergone network building with <code>build_domino()</code>
receptor	Name of a receptor active in at least one cell type in the domino object
ligands	Character vector of ligands capable of interaction with the receptor
ligand_expression_threshold	Minimum mean expression value of a ligand by a cell type for a chord to be rendered between the cell type and the receptor
cell_idents	Vector of cell types from cluster assignments in the domino object to be included in the plot.

Value

a data frame where each row describes plotting parameters of ligand-receptor interactions to pass to `render_circos_ligand_receptor()`

Examples

```
example(build_domino, echo = FALSE)  
#basic usage  
obtain_circos_expression(pbmc_dom_built_tiny, receptor = "CXCR3", ligands = "CCL20")
```

PBMC

PBMC RNAseq data subset

Description

A subset of the results of PBMC RNA-seq data.

Usage

```
data("PBMC")
```

Format

A list of::

RNA_count_tiny A subset of PBMC RNA-seq data: counts assay

RNA_zscore_tiny A subset of PBMC RNA-seq data: zscore assay

clusters_tiny A subset of PBMC RNA-seq data: clusters as defined by cell_type

Source

https://zenodo.org/records/10951634/files/pbmc3k_sce.rds

plot_differential_linkages

Plot differential linkages among domino results ranked by a comparative statistic

Description

Plot differential linkages among domino results ranked by a comparative statistic

Usage

```
plot_differential_linkages(
  differential_linkages,
  test_statistic,
  stat_range = c(0, 1),
  stat_ranking = c("ascending", "descending"),
  group_palette = NULL
)
```

Arguments

differential_linkages	a data frame output from the <code>test_differential_linkages()</code> function
test_statistic	column name of differential_linkages where the test statistic used for ranking linkages is stored (ex. 'p.value')
stat_range	a two value vector of the minimum and maximum values of test_statistic for plotting linkage features
stat_ranking	'ascending' (lowest value of test statistic is colored red and plotted at the top) or 'descending' (highest value of test statistic is colored red and plotted at the top).
group_palette	a named vector of colors to use for each group being compared

Value

A heatmap-class object of features ranked by test_statistic annotated with the proportion of subjects that showed active linkage of the features.

Examples

```
example(build_domino, echo = FALSE)
example(test_differential_linkages, echo = FALSE)
plot_differential_linkages(
  differential_linkages = tiny_differential_linkage_c1,
  test_statistic = "p.value",
  stat_ranking = "ascending"
)
```

```
print,domino-method    Print domino object
```

Description

Prints a summary of a domino object

Usage

```
## S4 method for signature 'domino'
print(x, ...)
```

Arguments

x	A domino object
...	Additional arguments to be passed to other methods

Value

A printed description of the number of cells and clusters in the domino object

Examples

```
example(build_domino, echo = FALSE)
print(pbmc_dom_built_tiny)
```

read_if_char	<i>Read in data if an object looks like path to it</i>
--------------	--

Description

Read in data if an object looks like path to it

Usage

```
read_if_char(obj)
```

Arguments

obj	object to read if not already object
-----	--------------------------------------

Value

Object itself or data read in from path

rename_clusters	<i>Renames clusters in a domino object</i>
-----------------	--

Description

This function renames the clusters used to build a domino object

Usage

```
rename_clusters(dom, clust_conv, warning = FALSE)
```

Arguments

dom	a domino object to rename clusters in
clust_conv	named vector of conversions from old to new clusters. Values are taken as new clusters IDs and names as old cluster IDs.
warning	logical. If TRUE, will warn if a cluster is not found in the conversion table. Default is FALSE.

Value

A domino object with clusters renamed in all applicable slots.

Examples

```
example(build_domino, echo = FALSE)
new_clust <- c("CD8_T_cell" = "CD8+ T Cells",
  "CD14_monocyte" = "CD14+ Monocytes", "B_cell" = "B Cells")
pbmc_dom_built_tiny <- rename_clusters(pbmc_dom_built_tiny, new_clust)
```

render_circos_ligand_receptor

Render Circos Ligand Receptor Plot

Description

Renders a circos plot from the output of `obtain_circos_expression()` to the active graphics device

Usage

```
render_circos_ligand_receptor(
  signaling_df,
  receptor,
  cell_colors = NULL,
  ligand_expression_threshold = 0.01
)
```

Arguments

<code>signaling_df</code>	Data frame output from <code>obtain_circos_expression()</code>
<code>receptor</code>	Name of a receptor active in at least one cell type in the domino object
<code>cell_colors</code>	Named vector of color names or hex codes where names correspond to the plotted cell types and the color values
<code>ligand_expression_threshold</code>	Minimum mean expression value of a ligand by a cell type for a chord to be rendered between the cell type and the receptor

Value

a circos plot is rendered to the active graphics device

Examples

```
example(build_domino, echo = FALSE)
#basic usage
circos_df <- obtain_circos_expression(pbmc_dom_built_tiny, receptor = "CXCR3", ligands = "CCL20")
render_circos_ligand_receptor(signaling_df = circos_df, receptor = "CXCR3")
```

resolve_complexes

Convert between complex names and gene names

Description

Convert between complex names and gene names

Usage

```
resolve_complexes(dom, genes)
```

Arguments

dom	A domino object
genes	A vector of genes, some of which may be complexes

Value

A list where any complexes are mapped to a vector of component genes. The list names are set to the input gene names.

resolve_names	<i>Convert between ligand names and gene names</i>
---------------	--

Description

Convert between ligand names and gene names

Usage

```
resolve_names(dom, genes)
```

Arguments

dom	A domino object
genes	A vector of genes on which to resolve ligand and gene names

Value

A vector of names where ligand names have been replaced with gene names if applicable

SCENIC	<i>SCENIC AUC subset</i>
--------	--------------------------

Description

A subset of SCENIC AUCs as applied to PBMC data.

Usage

```
data("SCENIC")
```

Format

A list of:

auc_tiny A subset of SCENIC AUCs

regulons_tiny A subset of SCENIC regulons

Source

<https://zenodo.org/records/10951634/files>

show,domino-method	<i>Show domino object information</i>
--------------------	---------------------------------------

Description

Shows content overview of domino object

Usage

```
## S4 method for signature 'domino'
show(object)
```

Arguments

object A domino object

Value

A printed description of cell numbers and clusters in the object

Examples

```
example(build_domino, echo = FALSE)
show(pbmcdom_built_tiny)
```

signaling_heatmap	<i>Create a network heatmap</i>
-------------------	---------------------------------

Description

Creates a heatmap of the signaling network. Alternatively, the network matrix can be accessed directly in the signaling slot of a domino object using the [dom_signaling\(\)](#) function.

Usage

```
signaling_heatmap(
  dom,
  clusts = NULL,
  min_thresh = -Inf,
  max_thresh = Inf,
  scale = "none",
  normalize = "none",
  ...
)
```

Arguments

dom	domino object with network built (build_domino())
clusts	vector of clusters to be included. If NULL then all clusters are used.
min_thresh	minimum signaling threshold for plotting. Defaults to -Inf for no threshold.
max_thresh	maximum signaling threshold for plotting. Defaults to Inf for no threshold.
scale	how to scale the values (after thresholding). Options are 'none', 'sqrt' for square root, or 'log' for log10.
normalize	options to normalize the matrix. Normalization is done after thresholding and scaling. Accepted inputs are 'none' for no normalization, 'rec_norm' to normalize to the maximum value with each receptor cluster, or 'lig_norm' to normalize to the maximum value within each ligand cluster
...	other parameters to pass to ComplexHeatmap::Heatmap()

Value

A heatmap rendered to the active graphics device

Examples

```
example(build_domino, echo = FALSE)
#basic usage
signaling_heatmap(pbmc_dom_built_tiny)
#scale
signaling_heatmap(pbmc_dom_built_tiny, scale = "sqrt")
#normalize
signaling_heatmap(pbmc_dom_built_tiny, normalize = "rec_norm")
```

signaling_network	<i>Create a cluster to cluster signaling network diagram</i>
-------------------	--

Description

Creates a network diagram of signaling between clusters. Nodes are clusters and directed edges indicate signaling from one cluster to another. Edges are colored based on the color scheme of the ligand expressing cluster

Usage

```
signaling_network(
  dom,
  cols = NULL,
  edge_weight = 0.3,
  clusts = NULL,
  showOutgoingSignalingClusts = NULL,
  showIncomingSignalingClusts = NULL,
  min_thresh = -Inf,
  max_thresh = Inf,
  normalize = "none",
  scale = "sq",
```

```

    layout = "circle",
    scale_by = "rec_sig",
    vert_scale = 3,
    plot_title = NULL,
    ...
)

```

Arguments

dom	a domino object with network built (<code>build_domino()</code>)
cols	named vector indicating the colors for clusters. Values are colors and names must match clusters in the domino object. If left as NULL then ggplot colors are generated for the clusters
edge_weight	weight for determining thickness of edges on plot. Signaling values are multiplied by this value
clusts	vector of clusters to be included in the network plot
showOutgoingSignalingClusts	vector of clusters to plot the outgoing signaling from
showIncomingSignalingClusts	vector of clusters to plot the incoming signaling on
min_thresh	minimum signaling threshold. Values lower than the threshold will be set to the threshold. Defaults to -Inf for no threshold
max_thresh	maximum signaling threshold for plotting. Values higher than the threshold will be set to the threshold. Defaults to Inf for no threshold
normalize	options to normalize the signaling matrix. Accepted inputs are 'none' for no normalization, 'rec_norm' to normalize to the maximum value with each receptor cluster, or 'lig_norm' to normalize to the maximum value within each ligand cluster
scale	how to scale the values (after thresholding). Options are 'none', 'sqrt' for square root, 'log' for log10, or 'sq' for square
layout	type of layout to use. Options are 'random', 'sphere', 'circle', 'fr' for Fruchterman-Reingold force directed layout, and 'kk' for Kamada Kawai for directed layout
scale_by	how to size vertices. Options are 'lig_sig' for summed outgoing signaling, 'rec_sig' for summed incoming signaling, and 'none'. In the former two cases the values are scaled with asinh after summing all incoming or outgoing signaling
vert_scale	integer used to scale size of vertices with or without variable scaling from size_verts_by.
plot_title	text for the plot's title.
...	other parameters to be passed to plot when used with an igraph object.

Value

An igraph plot rendered to the active graphics device

Examples

```
example(build_domino, echo = FALSE)
#basic usage
signaling_network(pbmc_dom_built_tiny, edge_weight = 2)
# scaling, thresholds, layouts, selecting clusters
signaling_network(
  pbmc_dom_built_tiny, showOutgoingSignalingClusts = "CD14_monocyte",
  scale = "none", norm = "none", layout = "fr", scale_by = "none",
  vert_scale = 5, edge_weight = 2)
```

summarize_linkages	<i>Summarize linkages from multiple domino objects</i>
--------------------	--

Description

Creates a `linkage_summary()` object storing the linkages learned in different domino objects as nested lists to facilitate comparisons of networks learned by domino across subject covariates.

Usage

```
summarize_linkages(domino_results, subject_meta, subject_names = NULL)
```

Arguments

<code>domino_results</code>	list of domino result with one domino object per subject. Names from the list must match <code>subject_names</code>
<code>subject_meta</code>	data frame that includes the subject features by which the objects could be grouped. The first column should must be subject names
<code>subject_names</code>	vector of subject names in <code>domino_results</code> . If <code>NULL</code> , defaults to first column of <code>subject_meta</code> .

Value

A linkage summary class object consisting of nested lists of the active transcription factors, active receptors, and incoming ligands for each cluster across multiple domino results

Examples

```
example(build_domino, echo = FALSE)

#create alternative clustering by shuffling cluster assignments
clusters_tiny_alt <- setNames(
  PBMC$clusters_tiny[c(121:240, 1:120, 241:360)],
  names(PBMC$clusters_tiny)
)
clusters_tiny_alt <- as.factor(clusters_tiny_alt)

#build an alternative domino object
pbmc_dom_tiny_alt <- create_domino(
  rl_map = rl_map_tiny,
  features = SCENIC$auc_tiny,
```

```

counts = PBMC$RNA_count_tiny,
z_scores = PBMC$RNA_zscore_tiny,
clusters = clusters_tiny_alt,
tf_targets = regulon_list_tiny,
use_clusters = TRUE,
use_complexes = TRUE,
remove_rec_dropout = FALSE
)

pbmc_dom_built_tiny_alt <- build_domino(
  dom = pbmc_dom_tiny_alt,
  min_tf_pval = .05,
  max_tf_per_clust = Inf,
  max_rec_per_tf = Inf,
  rec_tf_cor_threshold = .1,
  min_rec_percentage = 0.01
)

#create a list of domino objects
dom_ls <- list(
  dom1 = pbmc_dom_built_tiny,
  dom2 = pbmc_dom_built_tiny_alt
)

#compare the linkages across the two domino objects
meta_df <- data.frame("ID" = c("dom1", "dom2"), "group" = c("A", "B"))
summarize_linkages(
  domino_results = dom_ls, subject_meta = meta_df,
  subject_names = meta_df$ID
)

```

table_convert_genes	<i>Convert genes using a table</i>
---------------------	------------------------------------

Description

Takes a vector of gene inputs and a conversion table and returns a converted gene table

Usage

```
table_convert_genes(genes, from, to, conversion_table)
```

Arguments

genes	the genes to convert
from	gene symbol type of the input (ENSG, ENSMUSG, HGNC, MGI)
to	desired gene symbol type for the output (HGNC, MGI)
conversion_table	a data frame with column names corresponding to gene symbol types (mm.ens, hs.ens, mgi, hgnc) and rows corresponding to the gene symbols themselves

Value

A data frame of genes with original and corresponding converted symbols

test_differential_linkages

Statistical test for differential linkages across multiple domino results

Description

Statistical test for differential linkages across multiple domino results

Usage

```
test_differential_linkages(
  linkage_summary,
  cluster,
  group.by,
  linkage = "rec_lig",
  subject_names = NULL,
  test_name = "fishers.exact"
)
```

Arguments

linkage_summary	a linkage_summary() object
cluster	the name of the cell cluster being compared across multiple domino results
group.by	the name of the column in linkage_summary@subject_meta by which to group subjects for counting.
linkage	a stored linkage from the domino object. Can compare any of 'tfs', 'rec', 'incoming_lig', 'tfs_rec', or 'rec_lig'
subject_names	a vector of subject_names from the linkage_summary to be compared. If NULL, all subject_names in the linkage summary are included in counting.
test_name	the statistical test used for comparison. 'fishers.exact' : Fisher's exact test for the dependence of the proportion of subjects with an active linkage in the cluster on which group the subject belongs to in the group.by variable. Provides an odds ratio, p-value, and a Benjamini-Hochberg FDR-adjusted p-value (p.adj) for each linkage tested.

Value

A data frame of results from the test of the differential linkages. Rows correspond to each linkage tested. Columns correspond to:

- 'cluster' : the name of the cell cluster being compared
- 'linkage' : the type of linkage being compared
- 'group.by' : the grouping variable
- 'test_name' : the test used for comparison
- 'feature' : individual linkages compared
- 'test statistics' : test statistics provided are based on test method. 'fishers.exact' provides a odds ratio, p-value, and fdr-adjusted p-value.

- 'total_count' : total number of subjects where the linkage is active
- 'X_count' : number of subjects in each category of group.by (X) where the linkage is active
- 'total_n' : number of total subjects compared
- 'X_n' : total number of subjects in each category of group.by (X)

Examples

```
tiny_differential_linkage_c1 <- test_differential_linkages(  
  linkage_summary = mock_linkage_summary(), cluster = "C1", group.by = "group",  
  linkage = "rec", test_name = "fishers.exact"  
)
```

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