

Package ‘clustree’

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Type Package

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Description Deciding what resolution to use can be a difficult question when approaching a clustering analysis. One way to approach this problem is to look at how samples move as the number of clusters increases. This package allows you to produce clustering trees, a visualisation for interrogating clusterings as resolution increases.

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clustree-package	<i>Clustree</i>
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Description

Deciding what resolution to use can be a difficult question when approaching a clustering analysis. One way to approach this problem is to look at how samples move as the number of clusters increases. This package allows you to produce clustering trees, a visualisation for interrogating clusterings as resolution increases.

add_node_labels	<i>Add node labels</i>
-----------------	------------------------

Description

Add node labels to a clustering tree plot with the specified aesthetics.

Usage

```
add_node_labels(node_label, node_colour, node_label_size,  
  node_label_colour, node_label_nudge, allowed)
```

Arguments

node_label	the name of a metadata column for node labels
node_colour	either a value indicating a colour to use for all nodes or the name of a metadata column to colour nodes by
node_label_size	size of node label text
node_label_colour	colour of node_label text
node_label_nudge	numeric value giving nudge in y direction for node labels
allowed	vector of allowed node attributes to use as aesthetics

add_node_points	<i>Add node points</i>
-----------------	------------------------

Description

Add node points to a clustering tree plot with the specified aesthetics.

Usage

```
add_node_points(node_colour, node_size, node_alpha, allowed)
```

Arguments

node_colour	either a value indicating a colour to use for all nodes or the name of a metadata column to colour nodes by
node_size	either a numeric value giving the size of all nodes or the name of a metadata column to use for node sizes
node_alpha	either a numeric value giving the alpha of all nodes or the name of a metadata column to use for node transparency
allowed	vector of allowed node attributes to use as aesthetics

aggr_metadata	<i>Aggregate metadata</i>
---------------	---------------------------

Description

Aggregate a metadata column to get a summarized value for a cluster node

Usage

```
aggr_metadata(node_data, col_name, col_aggr, metadata, is_cluster)
```

Arguments

node_data	data.frame containing information about a set of cluster nodes
col_name	the name of the metadata column to aggregate
col_aggr	string naming a function used to aggregate the column
metadata	data.frame providing metadata on samples
is_cluster	logical vector indicating which rows of metadata are in the node to be summarized

Value

data.frame with aggregated data

assert_colour_node_aes	<i>Assert colour node aesthetics</i>
------------------------	--------------------------------------

Description

Raise error if an incorrect set of colour node parameters has been supplied.

Usage

```
assert_colour_node_aes(node_aes_name, prefix, metadata, node_aes,
  node_aes_aggr, min, max)
```

Arguments

node_aes_name	name of the node aesthetic to check
prefix	string indicating columns containing clustering information
metadata	data.frame containing metadata on each sample that can be used as node aesthetics
node_aes	value of the node aesthetic to check
node_aes_aggr	aggregation function associated with the node aesthetic
min	minimum numeric value allowed
max	maximum numeric value allowed

assert_node_aes	<i>Assert node aesthetics</i>
-----------------	-------------------------------

Description

Raise error if an incorrect set of node parameters has been supplied.

Usage

```
assert_node_aes(node_aes_name, prefix, metadata, node_aes, node_aes_aggr)
```

Arguments

node_aes_name	name of the node aesthetic to check
prefix	string indicating columns containing clustering information
metadata	data.frame containing metadata on each sample that can be used as node aesthetics
node_aes	value of the node aesthetic to check
node_aes_aggr	aggregation function associated with the node aesthetic

assert_numeric_node_aes	<i>Assert numeric node aesthetics</i>
-------------------------	---------------------------------------

Description

Raise error if an incorrect set of numeric node parameters has been supplied.

Usage

```
assert_numeric_node_aes(node_aes_name, prefix, metadata, node_aes,  
  node_aes_aggr, min, max)
```

Arguments

node_aes_name	name of the node aesthetic to check
prefix	string indicating columns containing clustering information
metadata	data.frame containing metadata on each sample that can be used as node aesthetics
node_aes	value of the node aesthetic to check
node_aes_aggr	aggregation function associated with the node aesthetic
min	minimum numeric value allowed
max	maximum numeric value allowed

build_tree_graph	<i>Build tree graph</i>
------------------	-------------------------

Description

Build a tree graph from a set of clusterings, metadata and associated aesthetics

Usage

```
build_tree_graph(clusterings, prefix, count_filter, prop_filter, metadata,
  node_aes_list)
```

Arguments

clusterings	numeric matrix containing clustering information, each column contains clustering at a separate resolution
prefix	string indicating columns containing clustering information
count_filter	count threshold for filtering edges in the clustering graph
prop_filter	in proportion threshold for filtering edges in the clustering graph
metadata	data.frame containing metadata on each sample that can be used as node aesthetics
node_aes_list	nested list containing node aesthetics

Value

`tidygraph::tbl_graph` object containing the tree graph

calc_sc3_stability	<i>Calculate SC3 stability</i>
--------------------	--------------------------------

Description

Calculate the SC3 stability index for every cluster at every resolution in a set of clusterings. The index varies from 0 to 1, where 1 suggests that a cluster is more stable across resolutions. See [calc_sc3_stability_cluster\(\)](#) for more details.

Usage

```
calc_sc3_stability(clusterings)
```

Arguments

clusterings	numeric matrix containing clustering information, each column contains clustering at a separate resolution
-------------	--

Value

matrix with stability score for each cluster

calc_sc3_stability_cluster	<i>Calculate single SC3 stability</i>
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Description

Calculate the SC3 stability index for a single cluster in a set of clusterings. The index varies from 0 to 1, where 1 suggests that a cluster is more stable across resolutions.

Usage

```
calc_sc3_stability_cluster(clusterings, res, cluster)
```

Arguments

clusterings	numeric matrix containing clustering information, each column contains clustering at a separate resolution
res	resolution of the cluster to calculate stability for
cluster	index of the cluster to calculate stability for

Details

This index was originally introduced in the SC3 package for clustering single-cell RNA-seq data. Clusters are awarded increased stability if they share the same samples as a cluster at another resolution and penalised at higher resolutions. We use a slightly different notation to describe the score but the results are the same:

$$s(c_{k,i}) = \frac{1}{size(L) + 1} \sum_{l \in L} \sum_{j \in N_l} \frac{size(c_{k,i} \cap c_{l,j})}{size(c_{l,j}) * size(N_l)^2}$$

Where:

- `c_{x, y}` is cluster `y` at resolution `x`
- `k` is the resolution of the cluster we want to score
- `i` is the index of the cluster we want to score
- `L` is the set of all resolutions except `k`
- `l` is a resolution in `L`
- `N_l` is the set of clusters at resolution `l` that share samples with `c_{k, i}`
- `j` is a cluster in `N_l`

Value

SC3 stability index

See Also

The documentation for the `calculate_stability` function in the SC3 package

check_node_aes_list	<i>Check node aes list</i>
---------------------	----------------------------

Description

Warn if node aesthetic names are incorrect

Usage

```
check_node_aes_list(node_aes_list)
```

Arguments

`node_aes_list` List of node aesthetics

Value

Corrected node aesthetics list

clustree	<i>Plot a clustering tree</i>
----------	-------------------------------

Description

Creates a plot of a clustering tree showing the relationship between clusterings at different resolutions.

Usage

```
clustree(x, ...)  
  
## S3 method for class 'matrix'  
clustree(x, prefix, suffix = NULL, metadata = NULL,  
  count_filter = 0, prop_filter = 0.1, layout = c("tree",  
    "sugiyama"), use_core_edges = TRUE, highlight_core = FALSE,  
  node_colour = prefix, node_colour_aggr = NULL, node_size = "size",  
  node_size_aggr = NULL, node_size_range = c(4, 15), node_alpha = 1,  
  node_alpha_aggr = NULL, node_text_size = 3,  
  scale_node_text = FALSE, node_text_colour = "black",  
  node_label = NULL, node_label_aggr = NULL, node_label_size = 3,  
  node_label_nudge = -0.2, edge_width = 1.5, edge_arrow = TRUE,  
  edge_arrow_ends = c("last", "first", "both"), show_axis = FALSE,  
  return = c("plot", "graph", "layout"), ...)  
  
## S3 method for class 'data.frame'  
clustree(x, prefix, ...)  
  
## S3 method for class 'SingleCellExperiment'  
clustree(x, prefix, exprs = "counts", ...)  
  
## S3 method for class 'seurat'  
clustree(x, prefix = "res.", exprs = c("data",  
  "raw.data", "scale.data"), ...)
```

Arguments

x	object containing clustering data
...	extra parameters passed to other methods
prefix	string indicating columns containing clustering information
suffix	string at the end of column names containing clustering information
metadata	data.frame containing metadata on each sample that can be used as node aesthetics
count_filter	count threshold for filtering edges in the clustering graph
prop_filter	in proportion threshold for filtering edges in the clustering graph

layout	string specifying the "tree" or "sugiyama" layout, see <code>igraph::layout_as_tree()</code> and <code>igraph::layout_with_sugiyama()</code> for details
use_core_edges	logical, whether to only use core tree (edges with maximum in proportion for a node) when creating the graph layout, all (unfiltered) edges will still be displayed
highlight_core	logical, whether to increase the edge width of the core network to make it easier to see
node_colour	either a value indicating a colour to use for all nodes or the name of a metadata column to colour nodes by
node_colour_aggr	if node_colour is a column name than a string giving the name of a function to aggregate that column for samples in each cluster
node_size	either a numeric value giving the size of all nodes or the name of a metadata column to use for node sizes
node_size_aggr	if node_size is a column name than a string giving the name of a function to aggregate that column for samples in each cluster
node_size_range	numeric vector of length two giving the maximum and minimum point size for plotting nodes
node_alpha	either a numeric value giving the alpha of all nodes or the name of a metadata column to use for node transparency
node_alpha_aggr	if node_alpha is a column name than a string giving the name of a function to aggregate that column for samples in each cluster
node_text_size	numeric value giving the size of node text if scale_node_text is FALSE
scale_node_text	logical indicating whether to scale node text along with the node size
node_text_colour	colour value for node text (and label)
node_label	additional label to add to nodes
node_label_aggr	if node_label is a column name than a string giving the name of a function to aggregate that column for samples in each cluster
node_label_size	numeric value giving the size of node label text
node_label_nudge	numeric value giving nudge in y direction for node labels
edge_width	numeric value giving the width of plotted edges
edge_arrow	logical indicating whether to add an arrow to edges
edge_arrow_ends	string indicating which ends of the line to draw arrow heads if edge_arrow is TRUE, one of "last", "first", or "both"
show_axis	whether to show resolution axis

return	string specifying what to return, either "plot" (a ggplot object), "graph" (a tbl_graph object) or "layout" (a ggraph layout object)
exprs	source of gene expression information to use as node aesthetics, for SingleCellExperiment objects it must be a name in assayNames(x), for a Seurat object it must be one of data, raw.data or scale.data

Details

Data sources

Plotting a clustering tree requires information about which cluster each sample has been assigned to at different resolutions. This information can be supplied in various forms, as a matrix, data.frame or more specialised object. In all cases the object provided must contain numeric columns with the naming structure PXS where P is a prefix indicating that the column contains clustering information, X is a numeric value indicating the clustering resolution and S is any additional suffix to be removed. For SingleCellExperiment objects this information must be in the colData slot and for Seurat objects it must be in the meta.data slot. For all objects except matrices any additional columns can be used as aesthetics, for matrices an additional metadata data.frame can be supplied if required.

Filtering

Edges in the graph can be filtered by adjusting the count_filter and prop_filter parameters. The count_filter removes any edges that represent less than that number of samples, while the prop_filter removes edges that represent less than that proportion of cells in the node it points towards.

Node aesthetics

The aesthetics of the plotted nodes can be controlled in various ways. By default the colour indicates the clustering resolution, the size indicates the number of samples in that cluster and the transparency is set to 100. Each of these can be set to a specific value or linked to a supplied metadata column. For a SingleCellExperiment or Seurat object the names of genes can also be used. If a metadata column is used then an aggregation function must also be supplied to combine the samples in each cluster. This function must take a vector of values and return a single value.

Layout

The clustering tree can be displayed using either the Reingold-Tilford tree layout algorithm or the Sugiyama layout algorithm for layered directed acyclic graphs. These layouts were selected as they are the algorithms available in the igraph package designed for trees. The Reingold-Tilford algorithm places children below their parents while the Sugiyama places nodes in layers while trying to minimise the number of crossing edges. See `igraph::layout_as_tree()` and `igraph::layout_with_sugiyama()` for more details. When `use_core_edges` is TRUE (default) only the core tree of the maximum proportion of edges for each node are used for constructing the layout. This can often lead to more attractive layouts where the core tree is more visible.

Value

a ggplot object (default), a tbl_graph object or a ggraph layout object depending on the value of return

Examples

```
data(iris_clusts)
clustree(iris_clusts, prefix = "K")
```

clustree_overlay	<i>Overlay a clustering tree</i>
------------------	----------------------------------

Description

Creates a plot of a clustering tree overlaid on a scatter plot of individual samples.

Usage

```
clustree_overlay(x, ...)

## S3 method for class 'matrix'
clustree_overlay(x, prefix, metadata, x_value, y_value,
  suffix = NULL, count_filter = 0, prop_filter = 0.1,
  node_colour = prefix, node_colour_aggr = NULL, node_size = "size",
  node_size_aggr = NULL, node_size_range = c(4, 15), node_alpha = 1,
  node_alpha_aggr = NULL, edge_width = 1, use_colour = c("edges",
  "points"), alt_colour = "black", point_size = 3, point_alpha = 0.2,
  point_shape = 18, label_nodes = FALSE, label_size = 3,
  plot_sides = FALSE, side_point_jitter = 0.45,
  side_point_offset = 1, ...)

## S3 method for class 'data.frame'
clustree_overlay(x, prefix, ...)

## S3 method for class 'SingleCellExperiment'
clustree_overlay(x, prefix, x_value,
  y_value, exprs = "counts", red_dim = NULL, ...)

## S3 method for class 'seurat'
clustree_overlay(x, x_value, y_value, prefix = "res.",
  exprs = c("data", "raw.data", "scale.data"), red_dim = NULL, ...)
```

Arguments

x	object containing clustering data
...	extra parameters passed to other methods
prefix	string indicating columns containing clustering information
metadata	data.frame containing metadata on each sample that can be used as node aesthetics
x_value	numeric metadata column to use as the x axis

y_value	numeric metadata column to use as the y axis
suffix	string at the end of column names containing clustering information
count_filter	count threshold for filtering edges in the clustering graph
prop_filter	in proportion threshold for filtering edges in the clustering graph
node_colour	either a value indicating a colour to use for all nodes or the name of a metadata column to colour nodes by
node_colour_aggr	if node_colour is a column name than a string giving the name of a function to aggregate that column for samples in each cluster
node_size	either a numeric value giving the size of all nodes or the name of a metadata column to use for node sizes
node_size_aggr	if node_size is a column name than a string giving the name of a function to aggregate that column for samples in each cluster
node_size_range	numeric vector of length two giving the maximum and minimum point size for plotting nodes
node_alpha	either a numeric value giving the alpha of all nodes or the name of a metadata column to use for node transparency
node_alpha_aggr	if node_aggr is a column name than a string giving the name of a function to aggregate that column for samples in each cluster
edge_width	numeric value giving the width of plotted edges
use_colour	one of "edges" or "points" specifying which element to apply the colour aesthetic to
alt_colour	colour value to be used for edges or points (whichever is NOT given by use_colour)
point_size	numeric value giving the size of sample points
point_alpha	numeric value giving the alpha of sample points
point_shape	numeric value giving the shape of sample points
label_nodes	logical value indicating whether to add labels to clustering graph nodes
label_size	numeric value giving the size of node labels is label_nodes is TRUE
plot_sides	logical value indicating whether to produce side on plots
side_point_jitter	numeric value giving the y-direction spread of points in side plots
side_point_offset	numeric value giving the y-direction offset for points in side plots
exprs	source of gene expression information to use as node aesthetics, for SingleCellExperiment objects it must be a name in assayNames(x), for a seurat object it must be one of data, raw.data or scale.data
red_dim	dimensionality reduction to use as a source for x_value and y_value

Details

Data sources

Plotting a clustering tree requires information about which cluster each sample has been assigned to at different resolutions. This information can be supplied in various forms, as a matrix, data.frame or more specialised object. In all cases the object provided must contain numeric columns with the naming structure PXS where P is a prefix indicating that the column contains clustering information, X is a numeric value indicating the clustering resolution and S is any additional suffix to be removed. For SingleCellExperiment objects this information must be in the colData slot and for Seurat objects it must be in the meta.data slot. For all objects except matrices any additional columns can be used as aesthetics.

Filtering

Edges in the graph can be filtered by adjusting the count_filter and prop_filter parameters. The count_filter removes any edges that represent less than that number of samples, while the prop_filter removes edges that represent less than that proportion of cells in the node it points towards.

Node aesthetics

The aesthetics of the plotted nodes can be controlled in various ways. By default the colour indicates the clustering resolution, the size indicates the number of samples in that cluster and the transparency is set to 100. Each of these can be set to a specific value or linked to a supplied meta-data column. For a SingleCellExperiment or Seurat object the names of genes can also be used. If a metadata column is used than an aggregation function must also be supplied to combine the samples in each cluster. This function must take a vector of values and return a single value.

Colour aesthetic

The colour aesthetic can be applied to either edges or sample points by setting use_colour. If "edges" is selected edges will be coloured according to the clustering resolution they originate at. If "points" is selected they will be coloured according to the cluster they are assigned to at the highest resolution.

Dimensionality reductions

For SingleCellExperiment and Seurat objects precomputed dimensionality reductions can be used for x or y aesthetics. To do so red_dim must be set to the name of a dimensionality reduction in reducedDimNames(x) (for a SingleCellExperiment) or x@dr (for a Seurat object). x_value and y_value can then be set to red_dimX when red_dim matches the red_dim argument and X is the column of the dimensionality reduction to use.

Value

a ggplot object if plot_sides is FALSE or a list of ggplot objects if plot_sides is TRUE

Examples

```
data(iris_clusts)
clustree_overlay(iris_clusts, prefix = "K", x_value = "PC1", y_value = "PC2")
```

get_tree_edges	<i>Get tree edges</i>
----------------	-----------------------

Description

Extract the edges from a set of clusterings

Usage

```
get_tree_edges(clusterings, prefix)
```

Arguments

clusterings	numeric matrix containing clustering information, each column contains clustering at a separate resolution
prefix	string indicating columns containing clustering information

Value

data.frame containing edge information

get_tree_nodes	<i>Get tree nodes</i>
----------------	-----------------------

Description

Extract the nodes from a set of clusterings and add relevant attributes

Usage

```
get_tree_nodes(clusterings, prefix, metadata, node_aes_list)
```

Arguments

clusterings	numeric matrix containing clustering information, each column contains clustering at a separate resolution
prefix	string indicating columns containing clustering information
metadata	data.frame containing metadata on each sample that can be used as node aesthetics
node_aes_list	nested list containing node aesthetics

Value

data.frame containing node information

iris_clusts	<i>Clustered Iris dataset</i>
-------------	-------------------------------

Description

Iris dataset clustered using k-means with a range of values of k

Usage

iris_clusts

Format

iris_clusts is a data.frame containing the normal iris dataset with additional columns holding k-means clusterings at different values of k and the first two principal components

Source

```
set.seed(1)
iris_mat <- as.matrix(iris[1:4])
iris_km <- sapply(1:5, function(x) {
  km <- kmeans(iris_mat, centers = x, iter.max = 100, nstart = 10)
  km$cluster
})
colnames(iris_km) <- paste0("K", 1:5)
iris_clusts <- cbind(iris, iris_km)
iris_pca <- prcomp(iris_clusts[1:4])
iris_clusts$PC1 <- iris_pca$x[, 1]
iris_clusts$PC2 <- iris_pca$x[, 2]
```

overlay_node_points	<i>Overlay node points</i>
---------------------	----------------------------

Description

Overlay clustering tree nodes on a scatter plot with the specified aesthetics.

Usage

```
overlay_node_points(nodes, x_value, y_value, node_colour, node_size,
  node_alpha)
```


Arguments

nodes	data.frame describing nodes
x_value	column of nodes to use for the x position
y_value	column of nodes to use for the y position
node_colour	either a value indicating a colour to use for all nodes or the name of a metadata column to colour nodes by
node_size	either a numeric value giving the size of all nodes or the name of a metadata column to use for node sizes
node_alpha	either a numeric value giving the alpha of all nodes or the name of a metadata column to use for node transparency

plot_overlay_side	<i>Plot overlay side</i>
-------------------	--------------------------

Description

Plot the side view of a clustree overlay plot. If the ordinary plot shows the tree from above this plot shows it from the side, highlighting either the x or y dimension and the clustering resolution.

Usage

```
plot_overlay_side(nodes, edges, points, prefix, side_value, graph_attr,
  node_size_range, edge_width, use_colour, alt_colour, point_size,
  point_alpha, point_shape, label_nodes, label_size, y_jitter, y_offset)
```

Arguments

nodes	data.frame describing nodes
edges	data.frame describing edges
points	data.frame describing points
prefix	string indicating columns containing clustering information
side_value	string giving the metadata column to use for the x axis
graph_attr	list describing graph attributes
node_size_range	numeric vector of length two giving the maximum and minimum point size for plotting nodes
edge_width	numeric value giving the width of plotted edges
use_colour	one of "edges" or "points" specifying which element to apply the colour aesthetic to
alt_colour	colour value to be used for edges or points (whichever is NOT given by use_colour)
point_size	numeric value giving the size of sample points
point_alpha	numeric value giving the alpha of sample points

point_shape	numeric value giving the shape of sample points
label_nodes	logical value indicating whether to add labels to clustering graph nodes
label_size	numeric value giving the size of node labels is label_nodes is TRUE
y_jitter	numeric value giving the y-direction spread of points in side plots
y_offset	numeric value giving the y-direction offset for points in side plots

Value

RETURN_DESCRIPTION

sc_example	<i>Simulated scRNA-seq dataset</i>
------------	------------------------------------

Description

A simulated scRNA-seq dataset generated using the splatter package and clustered using the SC3 and Seurat packages.

Usage

sc_example

Format

sc_example is a list holding a simulated scRNA-seq dataset. Items in the list included the simulated counts, normalised log counts, tSNE dimensionality reduction and cell assignments from SC3 and Seurat clustering.

Source

```
# Simulation
library("splatter") # Version 1.2.1

sim <- splatSimulate(batchCells = 200, nGenes = 10000,
                    group.prob = c(0.4, 0.2, 0.2, 0.15, 0.05),
                    de.prob = c(0.1, 0.2, 0.05, 0.1, 0.05),
                    method = "groups", seed = 1)
sim_counts <- counts(sim)[1:1000, ]

# SC3 Clustering
library("SC3") # Version 1.7.6
library("scater") # Version 1.6.2

sim_sc3 <- SingleCellExperiment(assays = list(counts = sim_counts))
rowData(sim_sc3)$feature_symbol <- rownames(sim_counts)
sim_sc3 <- normalise(sim_sc3)
```

```

sim_sc3 <- sc3(sim_sc3, ks = 1:8, biology = FALSE, n_cores = 1)
sim_sc3 <- runTSNE(sim_sc3)

# Seurat Clustering
library("Seurat") # Version 2.2.0

sim_seurat <- CreateSeuratObject(sim_counts)
sim_seurat <- NormalizeData(sim_seurat, display.progress = FALSE)
sim_seurat <- FindVariableGenes(sim_seurat, do.plot = FALSE,
                               display.progress = FALSE)
sim_seurat <- ScaleData(sim_seurat, display.progress = FALSE)
sim_seurat <- RunPCA(sim_seurat, do.print = FALSE)
sim_seurat <- FindClusters(sim_seurat, dims.use = 1:6,
                           resolution = seq(0, 1, 0.1),
                           print.output = FALSE)

sc_example <- list(counts = counts(sim_sc3),
                   tsne = reducedDim(sim_sc3),
                   sc3_clusters = colData(sim_sc3),
                   seurat_clusters = sim_seurat@meta.data)

```

store_node_aes

Store node aesthetics

Description

Store the names of node attributes to use as aesthetics as graph attributes

Usage

```
store_node_aes(graph, node_aes_list, metadata)
```

Arguments

graph	graph to store attributes in
node_aes_list	nested list containing node aesthetics
metadata	data.frame containing metadata that can be used as aesthetics

Value

graph with additional attributes

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