# Package 'CatsCradle'

December 30, 2024

**Title** This package provides methods for analysing spatial transcriptomics data and for discovering gene clusters

Version 1.1.0

**Description** This package addresses two broad areas. It allows for in-depth analysis of spatial transcriptomic data by identifying tissue neighbourhoods. These are contiguous regions of tissue surrounding individual cells. 'CatsCradle' allows for the categorisation of neighbourhoods by the cell types contained in them and the genes expressed in them. In particular, it produces Seurat objects whose individual elements are neighbourhoods rather than cells. In addition, it enables the categorisation and annotation of genes by producing Seurat objects whose elements are genes.

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```
aggregateFeatureMatrix
```

This function takes a matrix where rows are features and columns are cells, and a neighbourhood list, and creates an matrix where columns are the neighbourhoods, the rows are are the features and the values are aggregated expression values for cells in each neighbourhood.

#### Description

This function takes a matrix where rows are features and columns are cells, and a neighbourhood list, and creates an matrix where columns are the neighbourhoods, the rows are are the features and the values are aggregated expression values for cells in each neighbourhood.

# Usage

```
aggregateFeatureMatrix(M, nbhdList, aggregateFunction)
```

# Arguments

Μ	• a matrix where column names are cells and row names are features.
nbhdList	• a named list with memberships of the neighbourhoods of cells
aggregateFunctio	n
	• a function to aggregate expression (e.g. rowSums, rowMeans)

# Value

a matrix giving aggregated gene expression for a cell's neighbourhood.

aggregateGeneExpression

This function takes a Seurat object and a list of neighbourhoods and creates a Seurat object where the columns are the neighbourhoods, the rows are are the genes and the values are gene expression totals for the cells in each neighbourhood

# Description

This function takes a Seurat object and a list of neighbourhoods and creates a Seurat object where the columns are the neighbourhoods, the rows are are the genes and the values are gene expression totals for the cells in each neighbourhood

#### annotateGeneAsVector

# Usage

```
aggregateGeneExpression(
  f,
   neighbourhoods,
   verbose = TRUE,
   returnType = "Seurat"
)
```

# Arguments

f	• a Seurat object with layer counts or a SingleCellExperiment to be turned into a Seurat object
neighbourhoods	• Neighbourhoods as given by a collapsed expanded edge graph, as pro- duced by collapseNeighbourhoods. In particular, each cell should appear as nodeA.
verbose	• used to control trace, defaults to TRUE
returnType	• Will return a SingleCellExperiment if this is either of SCE, SingleCellExperiment or their lower-case equivalents. Otherwise, returns a Seurat object or SingleCellExperiment, depending on the parameter returnType.

#### Value

a Seurat object giving total gene expression in each neighbourhood or SingleCellExperiment

# Examples

```
getExample = make.getExample()
smallXenium = getExample('smallXenium',toy=TRUE)
extendedNeighbours = getExample('extendedNeighbours',toy=TRUE)
agg = aggregateGeneExpression(smallXenium,extendedNeighbours,verbose=FALSE)
```

annotateGeneAsVector	This function returns a numeric indicating which gene sets it does and
	does not belong to. This vector can be normalised to account for the
	sizes of the sets.

# Description

This function returns a numeric indicating which gene sets it does and does not belong to. This vector can be normalised to account for the sizes of the sets.

#### Usage

```
annotateGeneAsVector(gene, geneSets, normalise = FALSE)
```

# Arguments

gene	• the gene to annotate
geneSets	• a list of gene sets
normalise	• whether to normalise by set size

#### Value

a numeric

# Examples

```
hallmark = make.getExample()('hallmark')
Myc = annotateGeneAsVector('Myc',hallmark)
MycNormalised = annotateGeneAsVector('Myc',hallmark,TRUE)
```

annotateGenesByGeneSet

This function annotates genes with terms

#### Description

This essentially inverts a list of gene sets. It takes a list (e.g., Hallmark or GO) where each list item is a name of a gene set and gives the genes in that set and returns a list where each item is a gene and gives the gene sets that gene is in.

# Usage

```
annotateGenesByGeneSet(geneSets)
```

# Arguments

geneSets • a list of gene sets, e.g., as produced by readGmt

# Value

• A list where names are genes and values are lists of terms

#### Examples

```
hallmark = make.getExample()('hallmark')
annotatedGenes = annotateGenesByGeneSet(hallmark)
```

This takes a data frame of interaction counts as found by countLRInteractionsPerCell(), the underlying Seurat object and the neighbourhood Seurat object and annotates the counts with the cell type and the neighbourhood type corresponding to the cells of the interaction counts.

#### Description

This takes a data frame of interaction counts as found by countLRInteractionsPerCell(), the underlying Seurat object and the neighbourhood Seurat object and annotates the counts with the cell type and the neighbourhood type corresponding to the cells of the interaction counts.

#### Usage

annotateLRInteractionCounts(interactionCounts, obj, nbhdObj)

#### Arguments

interactionCounts	S
	• as found by countLRInteractionsPerCell()
obj	• a Seurat object, or SingleCellExperiment to be turned into a Seurat object
nbhd0bj	• a neighbourhood x cell type Seurat object or a SingleCellExperiment to be turned into a Seurat object

#### Value

This returns the interaction counts annotated with the cell type and neighbourhood type of each cell.

cellTypesPerCellTypeGraphFromCellMatrix This function converts a matrix as found by cellTypesPerCellType-Matrix into a directed igraph whose vertices correspond to seurat\_clusters and whose edge correspond to occupancy fraction.

# Description

This function converts a matrix as found by cellTypesPerCellTypeMatrix into a directed igraph whose vertices correspond to seurat\_clusters and whose edge correspond to occupancy fraction.

# Usage

```
cellTypesPerCellTypeGraphFromCellMatrix(
    M,
    colours = NULL,
    selfEdges = FALSE,
    minWeight = 0,
    edgeWeighting = 20,
    edgeCurved = 0.2,
    arrowSize = 4,
    arrowWidth = 4,
    plotGraph = TRUE
)
```

#### Arguments

М	• a matrix as found by cellTypesPerCellTypeMatrix. Note, however, that this matrix may need to be reduced to a square matrix as the matrix produced from a subset object may be missing certain cell types as rows.
colours	• a named vector of colours used to colour the vertices of the graph. The names are the seurat_clusters as character strings.
selfEdges	• a logical which determines whether to include self edges. Defaults to FALSE
minWeight	• Allows one to exclude edges of low weight. Defaults to 0, thus including all edges.
edgeWeighting	• a parameter used to thicken the edges in the display. Defaults to 20.
edgeCurved	• a parameter to set curvature of the edges. Defaults to 0.2
arrowSize	• a parameter to set arrow size. Defaults to 4.
arrowWidth	• a parameter to set arrow width. Defaults to 4.
plotGraph	• a logical which determines whether to plot the graph. Defaults to TRUE.

#### Value

This returns a directed igraph whose vertices are the cell types and whose arrows indicate "ownership" of cells of the target type by neighbourhoods of cells of the source type. Layout is done witht the FR algorithm and coordinates are found in the coords attribute of G. If colours were supplied these are found in color attribute of V(G). Edge weights and widths are found in the weight and width attributes of E(G).

# Examples

cellTypesPerCellTypeGraphFromNbhdMatrix

This function takes a neighbourhood-by-cell type matrix and produces a directed igraph showing the fractions of cells of each type in the neighbourhoods around cells of each type.

# Description

This function takes a neighbourhood-by-cell type matrix and produces a directed igraph showing the fractions of cells of each type in the neighbourhoods around cells of each type.

# Usage

```
cellTypesPerCellTypeGraphFromNbhdMatrix(
   nbhdByCellType,
   clusters,
   colours = NULL,
   selfEdges = FALSE,
   minWeight = 0,
   edgeWeighting = 20,
   edgeCurved = 0.2,
   arrowSize = 4,
   arrowWidth = 4,
   plotGraph = TRUE
)
```

#### Arguments

• A matrix whose rows are neighbourhoods each denoted by the cell at their center, whose columns are cell types, and whose entries are counts.
• a named vector whose names are the cells and whose entries are their seurat_clusters.
• a named vector of colours used to colour the vertices of the graph. The names are the seurat_clusters as character strings.
• a logical which determines whether to include self edges. Defaults to FALSE
• Allows one to exclude edges of low weight. Defaults to 0, thus including all edges.
• a parameter used to thicken the edges in the display. Defaults to 20.
• a parameter to set curvature of the edges. Defaults to 0.2
• a parameter to set arrow size. Defaults to 4.
• a parameter to set arrow width. Defaults to 4.
• a logical which determines whether to plot the graph. Defaults to TRUE.

#### Value

This returns a directed igraph whose vertices are the cell types and whose arrows indicate "ownership" of cells of the target type by neighbourhoods of cells of the source type. Layout is done witht the FR algorithm and coordinates are found in the coords attribute of G. If colours were supplied these are found in the color attribute of V(G). Edge weights and widths are found in the weight and width attributes of E(G).

collapseExtendedNBHDs This function takes an expanded neighbourhood list and collapses it to a nearest neighbourhood graph where all neighbours of degree <= n in the original graph are considered first neighbours.

#### Description

This function takes an expanded neighbourhood list and collapses it to a nearest neighbourhood graph where all neighbours of degree  $\leq n$  in the original graph are considered first neighbours.

#### Usage

```
collapseExtendedNBHDs(
    extendedNeighboursList,
    n = length(extendedNeighboursList)
)
```

# Arguments

n

extendedNeighboursList

- the results of getExtendedNBHDs()
- the maximum degree to connect neighbours. Defaults to the maximum degree neighbourhoods were expanded to in the results of getExtendedNBHDs().

# Value

a graph in neighbour format, i.e., a data frame with columns nodeA and nodeB, where nodes that were originally of degree <= n are connected.

#### Examples

```
extendedNeighboursList = make.getExample()('extendedNeighboursList',toy=TRUE)
extendedNeighbours = collapseExtendedNBHDs(extendedNeighboursList, 4)
```

combinatorialSpheres Discovers the combinatorial ball of a given radius around a fixed set of genes in the nearest neighbor graph of a Seurat object.

#### Description

Discovers the combinatorial ball of a given radius around a fixed set of genes in the nearest neighbor graph of a Seurat object.

#### Usage

combinatorialSpheres(NN, origin, radius)

#### Arguments

NN	• a nearest neighbors graph
origin	• a gene or list of genes
radius	• the radius of the combinatorial ball to be found.

#### Value

This returns a data frame whose columns are the gene name, the radius from the origin at which it is found

#### Examples

```
getExample = make.getExample()
NN = getExample('NN',toy=TRUE)
STranspose = getExample('STranspose',toy=TRUE)
spheres = combinatorialSpheres(NN,'Ccl6',3)
hallmark = getExample('hallmark')
geneSet = intersect(hallmark[["HALLMARK_TNFA_SIGNALING_VIA_NFKB"]],colnames(STranspose))
sphereAroundSet = combinatorialSpheres(NN,geneSet,1)
```

computeCellTypesPerCellTypeMatrix For each cell type, this function looks at the neighbourhoods around cells of that type and discovers the fractions of those cells of each type.

#### Description

For each cell type, this function looks at the neighbourhoods around cells of that type and discovers the fractions of those cells of each type.

```
computeCellTypesPerCellTypeMatrix(nbhdByCellType, cellTypes)
```

#### Arguments

nbhdByCellType	• A matrix whose rows are neighbourhoods each denoted by the cell at their center, whose columns are cell types, and whose entries are counts.
cellTypes	• named vector of cell types where names are each cell and cell types are a factor

# Value

A square matrix whose rownames and colnames are the seurat\_clusters as character strings. Each row corresponds to neighbourhoods around all cells of that type and the entries give the fractions of those neighbourhoods occupied by cells of each type.

#### Examples

```
getExample = make.getExample()
NBHDByCTMatrix = getExample('NBHDByCTMatrix')
clusters = getExample('clusters')
cellTypesPerCellType = computeCellTypesPerCellTypeMatrix(NBHDByCTMatrix,clusters)
```

computeEdgeGraph	This function takes a spatial graph and computes a new spatial graph where edges become nodes and A-B edges (in the original graph) be-
	come connected to all A- edges and all B- edges.

# Description

This function takes a spatial graph and computes a new spatial graph where edges become nodes and A-B edges (in the original graph) become connected to all A- edges and all B- edges.

#### Usage

```
computeEdgeGraph(spatialGraph, selfEdges = FALSE)
```

#### Arguments

spatialGraph	• a data frame of neighbouring edge pairs.
selfEdges	• a logical determining whether to include self edges. Defaults to False.

#### Value

a graph in neighbour format where edges in the original graph become nodes and A-B edges (in the original graph) become connected to all A- edges and all B- edges.

# Examples

```
delaunayNeighbours = make.getExample()('delaunayNeighbours')
edgeNeighbours = computeEdgeGraph(delaunayNeighbours)
```

computeEdgeObject This function takes interactionResults and creates a seural object where each point represents an edge between cells, and spatial coordinates are the centroids of edges between cells. The "expression matrix" is the binarised presence/absence of an interaction (ligand receptor pair) on an edge.

# Description

This function takes interactionResults and creates a seurat object where each point represents an edge between cells, and spatial coordinates are the centroids of edges between cells. The "expression matrix" is the binarised presence/absence of an interaction (ligand receptor pair) on an edge.

# Usage

```
computeEdgeObject(
  ligandReceptorResults,
  centroids,
  npcs = 10,
  returnType = "Seurat"
)
```

#### Arguments

ligandReceptorResults

0 1	<ul> <li>as returned by performLigandReceptorResultsAnalysis()</li> </ul>
centroids	• a dataframe containing centroids where rownames are cellnames and the first two columns contain x and y coordinates respectively.
npcs	• number of pcs used for PCA, defaults to 10
returnType	Determines whether to return a Seurat object or a SpatialExperiment. Will do the later if this is set to either SCE, SingleCellExperiment or lower case versions of either.

# Value

This returns a seural object where each point represents an edge between cells, and spatial coordinates are the centroids of edges between cells. The "expression matrix" is the binarised presence/absence of an interaction (ligand receptor pair) on an edge. Depending on the parameter returnType, this can alternatively be returned as a SpatialExperiment.

#### Examples

```
getExample = make.getExample()
centroids = getExample('centroids')
ligandReceptorResults = getExample('ligandReceptorResults')
edgeSeurat = computeEdgeObject(ligandReceptorResults, centroids)
```

computeGraphEmbedding This function adds a force directed graph embedding to a seurat object

# Description

This function adds a force directed graph embedding to a seurat object

#### Usage

```
computeGraphEmbedding(
  seuratObj,
  graph = defaultGraph(seuratObj),
  returnType = "Seurat"
)
```

#### Arguments

seurat0bj	• a seurat object of SingleCellExperiment to be turned into a Seurat object
graph	<ul> <li>which graph to extract. Defaults to paste0(f@active.assay,'_snn')</li> </ul>
returnType	• Will return a SingleCellExperiment if this is either of SCE, SingleCellExperiment or their lower-case equivalents. Otherwise, returns a Seurat object

# Value

a seurat object with a "graph" dimensionality reduction. Can also be a SingleCellExperiment depending on parameter returnType.

# Examples

```
NBHDByCTSeurat = make.getExample()('NBHDByCTSeurat',toy=TRUE)
objWithEmbedding = computeGraphEmbedding(NBHDByCTSeurat)
```

computeMoransI

This function takes a matrix where rows are features and columns are cells, and a neighbourhood list, and computes Moran's I.

#### Description

This function takes a matrix where rows are features and columns are cells, and a neighbourhood list, and computes Moran's I.

# Usage

computeMoransI(M, nbhdList)

#### Arguments

М	• a matrix where column names are cells and row names are features.
nbhdList	• a named list with memberships of the neighbourhoods of cells

# Value

a matrix giving aggregated gene expression for a cell's neighbourhood.

computeNBHDByCTMatrix	This function computes a matrix where neighbourhoods are rows and
	cell types are columns. The values in the matrix indicate the number
	of cells of a given type within a neighbourhood.

# Description

This function computes a matrix where neighbourhoods are rows and cell types are columns. The values in the matrix indicate the number of cells of a given type within a neighbourhood.

# Usage

```
computeNBHDByCTMatrix(spatialGraph, cellTypes)
```

# Arguments

spatialGraph	• a spatial graph in neighbour list format.
cellTypes	• named vector of cell types where names are each cell and cell types are a factor

# Value

a matrix of neighbourhoods by cell types

# Examples

```
getExample = make.getExample()
clusters = getExample('clusters')
delaunayNeighbours = getExample('delaunayNeighbours')
NBHDByCTMatrix = computeNBHDByCTMatrix(delaunayNeighbours,clusters)
```

computeNBHDVsCTObject This function creates a seurat object using a neighbourhood by cell type matrix

# Description

This function creates a seurat object using a neighbourhood by cell type matrix

#### Usage

```
computeNBHDVsCTObject(
  dataMatrix,
  resolution = 0.1,
  npcs = 10,
  n.neighbors = 30L,
  transpose = FALSE,
  verbose = TRUE,
  returnType = "Seurat"
)
```

# Arguments

dataMatrix	• a matrix of neighbourhoods by cell types or its transpose.
resolution	• resolution for clustering (default 0.1).
npcs	• number of pcs used for PCA, defaults to 10.
n.neighbors	• number of neighbors used by UMAP, defaults to 30.
transpose	• defaults to FALSE.
verbose	<ul> <li>defaults to TRUE, used to limit trace if FALSE</li> </ul>
returnType	• Will return a SingleCellExperiment if this is either of SCE, SingleCellExperiment or their lower-case equivalents. Otherwise, returns a Seurat object

#### Value

a seurat object based on a neighbourhood by cell type matrix or its transpose, containing clusters and UMAP. This can also be a SingleCellExperiment depending on the parameter returnType.

#### Examples

```
NBHDByCTMatrix = make.getExample()('NBHDByCTMatrix',toy=TRUE)
NBHDByCTSeurat = computeNBHDVsCTObject(NBHDByCTMatrix)
NBHDByCTSingleCell_sce = computeNBHDVsCTObject(NBHDByCTMatrix,returnType='SCE')
```

computeNeighbourEnrichment

This function calculates P values for whether cell types are more frequently neighbours than expected by chance. It does this by comparison to randomised neighbour graphs where edges are randomised but the degree of each node is preserved.

# Description

This function calculates P values for whether cell types are more frequently neighbours than expected by chance. It does this by comparison to randomised neighbour graphs where edges are randomised but the degree of each node is preserved.

# Usage

```
computeNeighbourEnrichment(
  spatialGraph,
  cellTypes,
  nSim = 1000,
  maxTries = 1000,
  verbose = TRUE
)
```

#### Arguments

spatialGraph	• a spatial graph in neighbour list format.
cellTypes	• named vector of cell types where names are each cell and cell types are a factor.
nSim	• the number of randomised graphs to create for pvalue calculation.
maxTries	• the maximum number of tries to remove self edges during graph randomi- sation. If self edges are remeining this will be reported.
verbose	• whether to print trace. Defaults to TRUE

#### Value

A square matrix containing upper tail p values describing whether two cell types are more frequently found together than expected by chance.

```
computeNeighboursDelaunay
```

This function computes a spatial graph where neighbors are identified based on Delaunay triangulation.

# Description

This function computes a spatial graph where neighbors are identified based on Delaunay triangulation.

#### Usage

computeNeighboursDelaunay(centroids)

#### Arguments

• a dataframe containing centroids where rownames are cellnames and the first two columns contain x and y coordinates respectively.

#### Value

a graph in neighbour format, i.e., a data frame with columns nodeA and nodeB.

# Examples

```
centroids = make.getExample()('centroids')
delaunayNeighbours = computeNeighboursDelaunay(centroids)
```

computeNeighboursEuclidean

This function computes a spatial graph where neighbors are identified based on euclidean distance and a user defined threshold.

#### Description

This function computes a spatial graph where neighbors are identified based on euclidean distance and a user defined threshold.

#### Usage

computeNeighboursEuclidean(centroids, threshold)

#### Arguments

centroids	• a dataframe containing centroids where rownames are cellnames and columns
	contain x and y coordinates respectively.
threshold	• a distance cut off to compute neighbours.

# Value

a graph in neighbour format, i.e., a data frame with columns nodeA and nodeB.

# Examples

```
centroids = make.getExample()('centroids')
euclideanNeighbours = computeNeighboursEuclidean(centroids,20)
```

countLRInteractionsPerCell

This function takes a listing of the neighbouring cells together with the presence or absence of each ligand-receptor pair on each edge and produces a count showing for each cell, how many neighbours it has with that interaction either as source or as target

#### Description

This function takes a listing of the neighbouring cells together with the presence or absence of each ligand-receptor pair on each edge and produces a count showing for each cell, how many neighbours it has with that interaction either as source or as target

#### Usage

```
countLRInteractionsPerCell(edges, sourceOrTarget)
```

#### Arguments

edges	• A data frame of neighbouring cells together with their interactions as pro- duced by getInteractionsOnEdges()
sourceOrTarget	• a character, either 'source' or 'target' telling which direction of interaction to count

#### Value

This returns a data frame with one row for each cell and a column giving the name of that cell and the other columns giving the counts of interactions that it has with its neighbours.

cullEdges

#### Description

This subsets edges by our chosen critera

# Usage

cullEdges(annEdges, cutoffSpec)

# Arguments

annEdges	• a data frame with columns nodeA, nodeB, length and cellTypePair as pro- duced by edgeLengthsAndCellTypePairs.
cutoffSpec	• This can be either a numeric value which will be applied across all edges as an upper limit or a data frame with columns cellTypePair and cutoff as produced by any of the edgeCutoffsBy functions

# Value

This returns a subset of the annotated edges

desymmetriseNN	This function takes the data frame of neighbor genes and reduces it
	so that each undirected edge is represented by only one directed edge.
	This ensures that randomisation does not magically split undirected
	edges into two edges.

# Description

This function takes the data frame of neighbor genes and reduces it so that each undirected edge is represented by only one directed edge. This ensures that randomisation does not magically split undirected edges into two edges.

# Usage

```
desymmetriseNN(NN)
```

# Arguments

NN

• a dataframe containing the neighborlist

# Value

• a neighborListDF with only one directed edge per undirected edge.

# Examples

```
NN = make.getExample()('NN',toy=TRUE)
print(dim(NN))
NNN = desymmetriseNN(NN)
print(dim(NNN))
```

directedHausdorfDistance

This finds the directed Hausdorf distance from A to B

# Description

This finds the directed Hausdorf distance from A to B

# Usage

```
directedHausdorfDistance(A, B)
```

#### Arguments

A	• an m x d matrix representing m points in dimension d
В	• an n x d matrix representing n points in dimension d

# Value

This returns the distance of the furthest point in A from its nearest point in B.

# Examples

```
A = matrix(seq_len(8),ncol=2)
B = matrix(seq(from=3,to=16),ncol=2)
d_hausdorf = directedHausdorfDistance(A,B)
```

edgeCutoffsByClustering

This finds proposed cutoffs for edge lengths by clustering the lengths of the edges for each cell type pair using k-means clustering with k = 2

# Description

This finds proposed cutoffs for edge lengths by clustering the lengths of the edges for each cell type pair using k-means clustering with k = 2

# Usage

edgeCutoffsByClustering(annEdges)

#### Arguments

annEdges	• a data frame with columns nodeA, nodeB, length and cellTypePair as pro-
	duced by edgeLengthsAndCellTypePairs.

#### Value

This returns a data frame with columns cellTypePair and cutoff.

# Examples

```
getExample = make.getExample()
centroids = getExample('centroids')
clusters = getExample('clusters')
delaunayNeighbours = getExample('delaunayNeighbours')
annEdges =
    edgeLengthsAndCellTypePairs(delaunayNeighbours,clusters,centroids)
cutoffDF = edgeCutoffsByClustering(annEdges)
```

edgeCutoffsByPercentile

This finds edge cutoffs by percentile

# Description

This finds edge cutoffs by percentile

#### Usage

```
edgeCutoffsByPercentile(annEdges, percentileCutoff)
```

# Arguments

annEdges

 a data frame with columns nodeA, nodeB, length and cellTypePair as produced by edgeLengthsAndCellTypePairs.

percentileCutoff

• a numeric

#### Value

This returns a data frame with columns cellTypePair and cutoff.

# Examples

```
getExample = make.getExample()
centroids = getExample('centroids')
clusters = getExample('clusters')
delaunayNeighbours = getExample('delaunayNeighbours')
annEdges =
    edgeLengthsAndCellTypePairs(delaunayNeighbours,clusters,centroids)
cutoffDF = edgeCutoffsByPercentile(annEdges,percentileCutoff=95)
```

edgeCutoffsByWatershed

This finds proposed cutoffs for edge lengths by computing the histogram of edge lengths for each cell type pair and then using the watershed algorithm to find the hump of the histogram containing the median.

# Description

This finds proposed cutoffs for edge lengths by computing the histogram of edge lengths for each cell type pair and then using the watershed algorithm to find the hump of the histogram containing the median.

# Usage

```
edgeCutoffsByWatershed(annEdges, nbins = 15, tolerance = 10)
```

# Arguments

annEdges	• a data frame with columns nodeA, nodeB, length and cellTypePair as pro- duced by edgeLengthsAndCellTypePairs.
nbins	• the number of bins for the histogram
tolerance	• the tolerance parameter for the watershed algorithm.

# Value

This returns a data frame with columns cellTypePair and cutoff.

# Examples

```
getExample = make.getExample()
centroids = getExample('centroids')
clusters = getExample('clusters')
delaunayNeighbours = getExample('delaunayNeighbours')
annEdges =
    edgeLengthsAndCellTypePairs(delaunayNeighbours,clusters,centroids)
cutoffDF = edgeCutoffsByWatershed(annEdges)
```

edgeCutoffsByZScore This finds edge cutoffs by z-score

# Description

This finds edge cutoffs by z-score

#### Usage

```
edgeCutoffsByZScore(annEdges, zCutoff)
```

# Arguments

annEdges	• a data frame with columns nodeA, nodeB, length and cellTypePair as pro- duced by edgeLengthsAndCellTypePairs.
zCutoff	• a numeric

# Value

This returns a data frame with columns cellTypePair and cutoff.

# edgeLengthPlot

# Examples

```
getExample = make.getExample()
centroids = getExample('centroids')
clusters = getExample('clusters')
delaunayNeighbours = getExample('delaunayNeighbours')
annEdges =
    edgeLengthsAndCellTypePairs(delaunayNeighbours,clusters,centroids)
cutoffDF = edgeCutoffsByZScore(annEdges,zCutoff=1.5)
```

edgeLengthPlot edgeLengthPlot

# Description

This plots histograms of the edge lengths broken out by the cell types of the cells they connect. It optionally plots a cutoff for each pair of types.

# Usage

```
edgeLengthPlot(annEdges, cutoffDF, whichPairs, xLim = 100, legend = FALSE)
```

# Arguments

annEdges	<ul> <li>A data frame as produced by edgeLengthsAndCellTypePairs</li> </ul>
cutoffDF	• A data frame with columns cellTypePair and cutoff. This defaults to NULL in which case no cutoffs will be plotted.
whichPairs	• Which cellTypePairs to plot. If this is NULL, we plot all pairs. If this is a numeric, we plot only pairs that have at least this many edges. If this is a character vector, we plot the pairs in this list.
xLim	• limits the extent of the plots. Defaults to 100. Can be set to NULL.
legend	Show legend, defaults to FALSE

#### Value

This returns a ggplot object

```
getExample = make.getExample()
centroids = getExample('centroids')
clusters = getExample('clusters')
delaunayNeighbours = getExample('delaunayNeighbours')
annEdges =
    edgeLengthsAndCellTypePairs(delaunayNeighbours,clusters,centroids)
cutoffDF = edgeCutoffsByPercentile(annEdges,95)
g = edgeLengthPlot(annEdges,cutoffDF,whichPairs=60)
```

```
edgeLengthsAndCellTypePairs
```

This function annotates edges with their distance and the types of cells they connect

# Description

This function annotates edges with their distance and the types of cells they connect

# Usage

```
edgeLengthsAndCellTypePairs(edges, clusters, centroids)
```

#### Arguments

edges	• A data frame with columns nodeA and nodeB giving the cells of each edge
clusters	• the clusters of each cell
centroids	• the centroids of each cell

#### Value

a data frame giving the edges (as nodeA and nodeB), their lengths and the cell type pair.

# Examples

```
getExample = make.getExample()
centroids = getExample('centroids')
clusters = getExample('clusters')
delaunayNeighbours = getExample('delaunayNeighbours')
annEdges = edgeLengthsAndCellTypePairs(delaunayNeighbours,clusters,centroids)
```

exampleObjects This returns the names of available example objects.

# Description

This returns the names of available example objects.

#### Usage

```
exampleObjects()
```

#### Value

A character vector of the names of available example data objects

# exSeuratObj

#### Examples

availableObjects = exampleObjects()

exSeuratObj exSeuratObj

#### Description

A Seurat object of 2000 genes by 540 cells.

#### Usage

exSeuratObj

#### Format

A Seurat object

A Seurat object of cells. It includes a UMAP of the cells and annotated clustering into cell types. It has been severely reduced in size to accommodate Bioconductor size restrictions.

#### Source

This is subset from the data associated with https://www.nature.com/articles/s41586-021-04006-z

```
geneSetsVsGeneClustersPValueMatrix
```

This compares the gene clusters to other gene sets e.g., GO, Hallmark, and determines the p-value for their overlaps when compared to a set of background genes.

# Description

This compares the gene clusters to other gene sets e.g., GO, Hallmark, and determines the p-value for their overlaps when compared to a set of background genes.

#### Usage

```
geneSetsVsGeneClustersPValueMatrix(
  geneSets,
  clusterDF,
  backgroundGenes,
  adjust = FALSE
)
```

#### Arguments

geneSets	• a named list of gene sets
clusterDF	• a data frame giving the cluster membership of each gene with columns gene and geneCluster
backgroundGenes	
	• a character vector of genes
adjust	• a logical deciding whether to adjust p values. Defaults to FALSE.

# Value

a matrix of p-values rows correspond to the gene sets and the columns correspond the the CatsCradle gene clusters

# Examples

getAverageExpressionDF

This converts an average gene expression matrix to a data frame.

# Description

This converts an average gene expression matrix to a data frame.

#### Usage

```
getAverageExpressionDF(M)
```

# Arguments

М

• An average gene expression matrix.

#### Value

A data frame with columns cellCluster, geneCluster and average expression

# getAverageExpressionMatrix

#### Examples

```
getExample = make.getExample()
averageExpMatrix = getExample('averageExpMatrix',toy=TRUE)
averageExpDF = getAverageExpressionDF(averageExpMatrix)
```

# getAverageExpressionMatrix

This computes average expression of each gene cluster in each cell cluster and returns the result as a matrix

# Description

This computes average expression of each gene cluster in each cell cluster and returns the result as a matrix

#### Usage

```
getAverageExpressionMatrix(
  f,
  fPrime,
  clusteringName = "seurat_clusters",
  layer = "scale.data"
)
```

#### Arguments

f	• The Seurat object of cells, or SingleCellExperiment to be turned into a Seurat object
fPrime	• The Seurat object of genes, or SingleCellExperiment to be turned into a Seurat object
clusteringName	In many cases, this will be the cell clustering, i.e., seurat_clusters, which is the default, but for neighbourhood Seurat objects, this can be neighbourhood_clusters.
layer	layer to use for expression values

#### Value

A matrix of the average expression where the rows correspond to cell clusters and the columns correspond to gene clusters.

```
getExample = make.getExample()
STranspose = getExample('STranspose',toy=TRUE)
exSeuratObj = getExample('exSeuratObj',toy=TRUE)
M = getAverageExpressionMatrix(exSeuratObj,STranspose,layer='data')
```

getBinarisedMatrix

This functions retrieves an expression matrix from a seural object or SingleCellExperiment and binarises it.

# Description

This functions retrieves an expression matrix from a seural object or SingleCellExperiment and binarises it.

#### Usage

getBinarisedMatrix(obj, cutoff = 0, layer = "count")

#### Arguments

obj	• a Seurat object or SingleCellExperiment to be turned into a Seurat object
cutoff	• a cutoff for binarisation. Defaults to 0.
layer	• layer to fetch data from. Defaults to count.

# Value

A binarised expression matrix where rows are genes and columns are cells.

getClusterOrder This gets the clusters in their cannonical order

# Description

This deals with skullduggery in which seurat\_clusters has been converted from a factor to a character or a numeric.

#### Usage

getClusterOrder(f)

#### Arguments

f

 a Seurat object with meta.data column seurat\_clusters or SingleCellExperiment to be turned into a Seurat object

# Value

A vector of these unique values in order

```
STranspose = make.getExample()('STranspose',toy=TRUE)
geneClusters = getClusterOrder(STranspose)
```

getExtendedNBHDs This function takes a nearest neighbour graph and a radius and calculates nth degree neighbour graphs where max(n) == radius

# Description

This function takes a nearest neighbour graph and a radius and calculates nth degree neighbour graphs where max(n) == radius

#### Usage

```
getExtendedNBHDs(spatialGraph, n)
```

#### Arguments

spatialGraph n a nearest neighbour graph the maximum degree to calculate a neighbour graph with edges connecting

#### Value

A named list of neighbour graphs, where each graph contains edges connecting vertices of degree n. Each graph is named according to degree n.

#### Examples

```
delaunayNeighbours = make.getExample()('delaunayNeighbours')
extendedNeighboursList = getExtendedNBHDs(delaunayNeighbours, 4)
```

vertices of degree n for.

getFeatureZScores This gets z-scores for the values of features

#### Description

This gets z-scores for the values of features

#### Usage

```
getFeatureZScores(f, features = rownames(f), layer = "data")
```

# Arguments

f	• a Seurat object of cells or SingleCellExperiment to be converted to a Seurat object
layer	• the data layer to retrieve
featurs	• a set of features to retrieve z-scores for, defaults to rownames(f)

# Value

This returns a data frame with a column for each feature and a row for each cell

# Examples

```
getExample = make.getExample()
exSeuratObj = getExample('exSeuratObj',toy=TRUE)
df = getFeatureZScores(exSeuratObj)
```

getGeneClusterAveragesPerCell

This produces a matrix giving the average expression of gene clusters in cells. By default, it uses all cells and all gene clusters.

# Description

This produces a matrix giving the average expression of gene clusters in cells. By default, it uses all cells and all gene clusters.

#### Usage

```
getGeneClusterAveragesPerCell(
    f,
    fPrime,
    cells = colnames(f),
    geneClusters = getClusterOrder(fPrime),
    layer = "data"
)
```

# Arguments

f	• the cell Seurat object or SingleCellExperiment to be turned into a Seurat object
fPrime	• the genes Seurat object or SingleCellExperiment to be turned into a Seurat object
cells	• the cells to compute this for
geneClusters	• the geneClusters to compute average expression for
layer	• the data layer to use, defaults to 'data'

# Value

A matrix where the rows correspond to cells, the columns correspond to geneClusters and the entries give average expression for each cluster in each cell

# getGeneNeighbors

# Examples

```
getExample = make.getExample()
exSeuratObj = getExample('exSeuratObj',toy=TRUE)
STranspose = getExample('STranspose',toy=TRUE)
clusterExpression = getGeneClusterAveragesPerCell(exSeuratObj,STranspose)
```

getGeneNeighbors	This function gets the neighbors of a given gene using either the gene
	Seurat object or its nearest neighbor graph returned from getNearest-
	NeighbourLists

# Description

This function gets the neighbors of a given gene using either the gene Seurat object or its nearest neighbor graph returned from getNearestNeighbourLists

#### Usage

getGeneNeighbors(gene, NN)

# Arguments

gene	• the gene in question
NN	• either the gene Seurat object or its nearest neighbor graph as found by getN- earestNeighbourLists. This can also be a SingleCellExperiment which will be converted to a Seurat object

# Value

the neighboring genes

```
library(Seurat)
getExample = make.getExample()
STranspose = getExample('STranspose',toy=TRUE)
NN = getExample('NN',toy=TRUE)
neighbors = getGeneNeighbors("Ccl6",STranspose)
neighborsAgain = getGeneNeighbors("Ccl6",NN)
```

#### getInteractionsOnEdges

This function takes a binarised expression matrix, a set of ligand receptor pairs and a set of edges denoting neighbouring cells and annotates these with the ligand receptor interactions taking place on those edges in each direction.

#### Description

This function takes a binarised expression matrix, a set of ligand receptor pairs and a set of edges denoting neighbouring cells and annotates these with the ligand receptor interactions taking place on those edges in each direction.

#### Usage

getInteractionsOnEdges(M, pairDF, spatialGraph)

#### Arguments

М	• a binarised expression matrix where rows are genes and columns are cells.
pairDF	• a data frame giving the ligand-receptor pairs
spatialGraph	• a data frame of neighbouring cell pairs. Note that each row is a directed edge (A,B) so that this data frame should have both the edge (A,B) and the edge (B,A)

#### Value

This returns a data frame whose first two columns give the neighbouring cells. Each of the remaining columns is a logical corresponding to a ligand-receptor pair telling whether the ligand is expressed in the first cell and the receptor is expressed in the second cell.

getLigandReceptorNetwork

This function retrieves the Nichenetr ligand- receptor network for mouse or human.

# Description

This function retrieves the Nichenetr ligand- receptor network for mouse or human.

#### Usage

getLigandReceptorNetwork(species)

#### Arguments

species

• either 'human' or 'mouse'

#### Value

This returns a data frame whose first two columns are from and to, i.e., ligand and receptor. These are derived from the nichenetr ligand receptor networks.

#### Examples

lrn = getLigandReceptorNetwork('human')

```
getLigandReceptorPairsInPanel
```

This functions takes an Seurat object, its species and a ligand receptor network and subsets the ligand receptor network to those pairs that occur in the panel

# Description

This functions takes an Seurat object, its species and a ligand receptor network and subsets the ligand receptor network to those pairs that occur in the panel

# Usage

```
getLigandReceptorPairsInPanel(
   obj,
   species,
   lrn = getLigandReceptorNetwork(species)
)
```

#### Arguments

obj	• a Seurat object or SingleCellExperiment to be converted to a Seurat object
species	• either 'human' or 'mouse'
lrn	• a ligand-receptor network, i.e., a data frame with columns from and to. By default, it retrieves the nichenetr ligand receptor network

#### Value

This returns a data frame with columns ligand and receptor

```
smallXenium = make.getExample()('smallXenium')
lrPairs = getLigandReceptorPairsInPanel(smallXenium, "mouse")
```

getNearbyGenes Nearby genes

# Description

This finds the genes near a give subset using either a dimensional reduction or the nearest neighbor graph

# Usage

```
getNearbyGenes(
   fPrime,
   geneSet,
   radius,
   metric = "umap",
   numPCs = NULL,
   weights = FALSE
)
```

# Arguments

fPrime	<ul> <li>a Seurat object of genes or SingleCellExperiment to be converted to a Seurat object</li> </ul>
geneSet	• set of genes
radius	• the distance around the given set
metric	• the metric to use, one of umap, tsne, pca or nearest neighbor
numPCs	• used only if the metric is pca
weights	• whether to use edge weights in the NN case

#### Value

This returns a named vector whose values are distance from geneSet and whose names are the nearby genes.

```
getExample = make.getExample()
STranspose = getExample('STranspose',toy=TRUE)
hallmark = getExample('hallmark')
geneSet = intersect(colnames(STranspose),hallmark[["HALLMARK_TNFA_SIGNALING_VIA_NFKB"]])
geometricallyNearby = getNearbyGenes(STranspose,geneSet,radius=0.2,metric='umap')
combinatoriallyNearby = getNearbyGenes(STranspose,geneSet,radius=1,metric='NN')
weightedNearby = getNearbyGenes(STranspose,'Myc',radius=1,metric='NN',weights=TRUE)
```

getNearestNeighbourLists

This function extracts a shared nearest neighbor network from a Seurat object

#### Description

This function extracts a shared nearest neighbor network from a Seurat object

#### Usage

```
getNearestNeighbourLists(f, graph = defaultGraph(f))
```

# Arguments

f	• a Seurat object or SingleCellExperiment to be converted to a Seurat object
graph	<ul> <li>which graph to extract. Defaults to paste0(f@active.assay,'_snn')</li> </ul>

# Value

• This returns dataframe of neighbors: nodeA - node names for node A nodeB - node names for node B weight - edge weight

#### Examples

STranspose = make.getExample()('STranspose',toy=TRUE)
NN = getNearestNeighbourLists(STranspose)

#### getObjectSubsetClusteringPValue

This function computes a p-value for the geometric clustering of a gene set (in UMAP or PCA reduction) based on the median distance from its complement to the set.

#### Description

This function computes a p-value for the geometric clustering of a gene set (in UMAP or PCA reduction) based on the median distance from its complement to the set.

#### Usage

```
getObjectSubsetClusteringPValue(
  fPrime,
  geneSubset,
  numTrials = 1000,
  reduction = "UMAP",
  numPCs = 10
)
```

# Arguments

fPrime	• a transposed Seurat object, i.e. a Seurat object of genes or SingleCellExperiment to be converted to a Seurat object
geneSubset	• a subset of the genes which can be given as a character vector as a logical vector
numTrials	• the number of random trials to be carried out for randomised testing. Defaults to 1000.
reduction	• can be 'UMAP' or 'PCA', defaults to 'UMAP'
numPCs	• number of PCs to use if reduction is 'PCA'

# Value

A p-value reporting how often a random subset of the same size is sufficiently clustered to produce an equally large distance from its complement.

# Examples

```
getExample = make.getExample()
STranspose = getExample('STranspose')
hallmark = getExample('hallmark',toy=TRUE)
geneSubset = intersect(colnames(STranspose),hallmark[["HALLMARK_TNFA_SIGNALING_VIA_NFKB"]])
p = getObjectSubsetClusteringPValue(STranspose,geneSubset,100)
```

#### getObjectSubsetClusteringStatistics

This function computes statistics for the geometric clustering of a gene set (in UMAP or PCA reduction) based on the median distance from its complement to the set.

# Description

This function computes statistics for the geometric clustering of a gene set (in UMAP or PCA reduction) based on the median distance from its complement to the set.

#### Usage

```
getObjectSubsetClusteringStatistics(
    fPrime,
    geneSubset,
    numTrials = 1000,
    reduction = "UMAP",
    numPCs = 10
)
```

# humanLRN

# Arguments

fPrime	• a transposed Seurat object, i.e. a Seurat object of genes or SingleCellExperiment to be converted to a Seurat object
geneSubset	• a subset of the genes which can be given as a character vector or as a logical vector
numTrials	• the number of random trials to be carried out for randomised testing. Defaults to 1000.
reduction	• can be 'UMAP' or 'PCA', defaults to 'UMAP'
numPCs	• number of PCs to use if reduction is 'PCA'

#### Value

A list of statistics resulting from the testing of randomised subsets of the same size as the given gene subset. These include subsetDistance, the actual median complement distance; randomSubsetDistance, the median complement distances for randomised subsets; pValue, computed by comparing the real and randomised distances; and zScore, the z-distance of the actual median distance from the mean of the randomised distances.

#### Examples

```
getExample = make.getExample()
STranspose = getExample('STranspose',toy=TRUE)
hallmark = getExample('hallmark')
geneSubset = intersect(colnames(STranspose),hallmark[["HALLMARK_TNFA_SIGNALING_VIA_NFKB"]])
stats = getObjectSubsetClusteringStatistics(STranspose,geneSubset,100)
```

humanLRN humanLRN

# Description

A data frame giving 12019 human ligand receptor pairs

#### Usage

humanLRN

# Format

a data frame with two columns, 'from' and 'to'

A data frame with two columns, 'from' and 'to'. Each row represents a human ligand - receptor pair.

# Source

This is taken from the nichenetr package, url = https://www.nature.com/articles/s41592-019-0667-5. Specifically we use the human ligand - receptor network. ligandReceptorResults ligandReceptorResults

#### Description

The result of performLigandReceptorAnalysis(smallXenium, delaunayNeighbours, "mouse", clusters,verbose=FALSE)

#### Usage

ligandReceptorResults

# Format

A list of data frames.

A list containing: interactionsOnEdges - a data frame whose first two columns give the neighbouring cells and next two columns give their corresponding clusters. Each of the remaining columns is a logical corresponding to a ligand-receptor pair telling whether the ligand is expressed in the first cell and the receptor is expressed in the second cell. totalInteractionsByCluster - a dataframe where the first column gives a directed (sender-receiver) pair of clusters. The second column gives the total number of edges between those clusters. The remaining columns give the total numbers of edges on which particular ligand receptor interactions are present. meanInteractionsByCluster - a dataframe where the first column gives a directed (sender-receiver) pair of clusters. The second column gives the total number of edges between those clusters. The remaining columns give the total numbers of edges on which particular ligand receptor interactions are present (for that cluster pair) divided by the total number of edges between those clusters. simResults - a dataframe where the rownames are sender-receiver cluster pairs and column names are ligand receptor pairs. Values give the number of simulations for which observed values are greater than simulated values. pValues - a dataframe where the rownames are sender-receiver cluster pairs and column names are ligand receptor pairs. Entries are uppertail pvalues describing whether a particular ligand receptor interaction is observed more frequently between 2 clusters than expected.

### Source

Created from smallXenium and delaunayNeighbours by using performLigandReceptorAnalysis(()

make.getExample

*This function makes the function which retrieves and makes example data objects.* 

# Description

This function makes the function which retrieves and makes example data objects.

# Usage

make.getExample()

# Value

This returns the function which retrieves and makes example data objects. The latter saves any object it has found for quicker return. Using the value 'list' causes it to return the list of all objects found so far.

# Examples

```
getExample = make.getExample()
## Provided:
smallXenium = getExample('smallXenium')
## Computed:
delaunayNeighbours = getExample('delaunayNeighbours')
```

```
makeLRInteractionHeatmap
```

*This function takes ligandReceptorResults and plots a heatmap of - log10(pvalues).* 

# Description

This function takes ligandReceptorResults and plots a heatmap of -log10(pvalues).

# Usage

```
makeLRInteractionHeatmap(
    ligandReceptorResults,
    clusters,
    colours = c(),
    pValCutoffClusterPair = 0.05,
    pValCutoffLigRec = 0.05,
    labelClusterPairs = TRUE
)
```

# Arguments

ligandReceptorResults	

	<ul> <li>as returned by performLigandReceptorAnalysis()</li> </ul>
clusters	• named vector of cell types where names are each cell and clusters are a factor
colours	• a named list of colours where names are clusters. If not specified the default pheatmap colour scheme will be used.

• a cutoff for showing interactions between two clusters. A cluster pair must
have at least one ligand-receptor interaction pvalue < pValCutoffCluster-
Pair. Defaults to 0.05.

#### pValCutoffLigRec

pValCutoffClusterPair

• a cutoff for showing interactions between a ligand and receptor. At least one cluster pair must have pvalue < pValCutoffLigRec for ligand-receptor pair. Defaults to 0.05.

labelClusterPairs

• show labels for cluster pairs. Defaults to TRUE.

#### Value

matrix of -log10(pvalues) that underlies the heatmap.

# Examples

```
getExample = make.getExample()
clusters = getExample('clusters')
colours = getExample('colours')
ligandReceptorResults = getExample('ligandReceptorResults')
ligRecMatrix = makeLRInteractionHeatmap(ligandReceptorResults,
clusters, colours = colours, labelClusterPairs = FALSE)
```

makeSummedLRInteractionHeatmap

This function takes ligandReceptorResults and plots a heatmap of the total number of ligand receptor interactions between clusters.

# Description

This function takes ligandReceptorResults and plots a heatmap of the total number of ligand receptor interactions between clusters.

#### Usage

```
makeSummedLRInteractionHeatmap(
    ligandReceptorResults,
    clusters,
    type,
    logScale = TRUE
)
```

# Arguments

ligandReceptorResults	
	<ul> <li>as returned by performLigandReceptorAnalysis()</li> </ul>
clusters	• named vector of cell types where names are each cell and clusters are a factor
type	• "total" or "mean" to plot raw total interactions or mean interactions per edge.
logScale	• plot heatmap using log scale (defaults to TRUE)

# Value

matrix of total ligand receptor interactions that underlies t he heatmap.

# Examples

```
getExample = make.getExample()
clusters = getExample('clusters')
ligandReceptorResults = getExample('ligandReceptorResults')
cellTypePerCellTypeLigRecMatrix =
makeSummedLRInteractionHeatmap(ligandReceptorResults, clusters, "mean")
```

meanGeneClusterOnCellUMAP

Mean gene cluster on cell umap

# Description

This function paints gene expression for a given gene cluster on cell umap.

# Usage

```
meanGeneClusterOnCellUMAP(f, fPrime, geneCluster)
```

# Arguments

f	<ul> <li>a Seurat object of cells or SingleCellExperiment to be converted to a Seurat object</li> </ul>
fPrime	• the corresponding Seurat object of genes SingleCellExperiment to be converted to a Seurat object
geneCluster	• a gene cluster of fPrime

# Value

This returns a ggplot object

# Examples

```
getExample = make.getExample()
exSeuratObj = getExample('exSeuratObj',toy=TRUE)
STranspose = getExample('STranspose',toy=TRUE)
g = meanGeneClusterOnCellUMAP(exSeuratObj,STranspose,geneCluster=0)
```

meanZPerCluster

This finds the mean z-score for features in subsets of cells e.g., in each of the seurat\_clusters

# Description

This finds the mean z-score for features in subsets of cells e.g., in each of the seurat\_clusters

# Usage

```
meanZPerCluster(f, features, clusterBy = "seurat_clusters", layer = "data")
```

# Arguments

f	• a Seurat object of cells or SingleCellExperiment to be converted to a Seurat object
features	• a set of features of f
clusterBy	• the name of the column of f@meta.data to be used to subset the cells
layer	• the data layer to be used for z-scores

#### Value

This returns a data frame each of whose columns corresponds to a value of the clusterBy data. In the case where the clusterBy data is a factor or numeric, it prepends cluster\_ to the column name.

### Examples

meanZPerClusterOnUMAP This collects together mean z-score data together with UMAP coordinates from the gene seurat object for plotting.

#### Description

This collects together mean z-score data together with UMAP coordinates from the gene seurat object for plotting.

#### Usage

```
meanZPerClusterOnUMAP(f, fPrime, clusterBy = "seurat_clusters", layer = "data")
```

#### Arguments

f	• a Seurat object of cells or SingleCellExperiment to be converted to a Seurat object
fPrime	• the corresponding Seurat object of genes SingleCellExperiment to be converted to a Seurat object
clusterBy	• the name of the column of f@meta.data to be used to subset the cells
layer	• the data layer to be used for z-scores

# Value

This returns a data frame with the UMAP coordinates of the gene Seurat object and the average zscore for each gene within each of the cell clusters defined by the clusterBy column of the meta.data of f.

#### Examples

```
getExample = make.getExample()
exSeuratObj = getExample('exSeuratObj',toy=TRUE)
STranspose = getExample('STranspose',toy=TRUE)
df = meanZPerClusterOnUMAP(exSeuratObj,STranspose,clusterBy='shortName')
```

medianComplementDistance

This takes a set S of n points in dimension d given by an n x d matrix and a subset A given by a logical and returns the median distance from the complement to the given subset.

#### Description

This takes a set S of n points in dimension d given by an n x d matrix and a subset A given by a logical and returns the median distance from the complement to the given subset.

#### Usage

medianComplementDistance(S, idx)

# Arguments

S	• an n x d matrix representing a set of n points in dimension d
idx	• a logical of length n representing a subset of S. This should not be the empty set or all of S.

# Value

This returns the median distance from the complement to the subset

# Examples

```
S = matrix(seq_len(12),ncol=2)
idx = c(rep(FALSE,3),rep(TRUE,3))
compDist = medianComplementDistance(S,idx)
```

medianComplementPValue

This takes a set S of n points in dimension d and a subset A and computes a p-value for the co-localization of the subset by comparing the median complement distance for the given set to values of the median complement distance computed for random subsets of the same size.

# Description

This takes a set S of n points in dimension d and a subset A and computes a p-value for the colocalization of the subset by comparing the median complement distance for the given set to values of the median complement distance computed for random subsets of the same size.

# Usage

```
medianComplementPValue(S, idx, numTrials = 1000, returnTrials = FALSE)
```

# Arguments

S	• an n x d matrix representing a set of n points in dimension d
idx	• a logical of length n representing a subset of S. This should not be the empty set or all of S.
numTrials	• the number of random trials to perform, defaults to 1000
returnTrials	• whether to report the real and random median complement distances.

# moransI

# Value

By default this reports a p-value. If returnTrials is set, this returns a list giving the p-value, the actual complement distance and the random complement distances.

#### Examples

```
library(Seurat)
getExample = make.getExample()
STranspose = getExample('STranspose',toy=TRUE)
hallmark = getExample('hallmark')
S = data.matrix(FetchData(STranspose,c('umap_1','umap_2')))
idx = colnames(STranspose) %in% hallmark[["HALLMARK_TNFA_SIGNALING_VIA_NFKB"]]
mcpv = medianComplementPValue(S,idx,numTrials=100)
```

moransI

moransI

# Description

A data fame containing Moran's I and related pvalues.

#### Usage

moransI

# Format

A data fame containing Moran's I and related pvalues.

Moran's I values calculated for the genes in smallXenium (using the SCT assay). Pvalues derived using 100 permutations.

# Source

Created from smallXenium and delaunayNeighbours by using runMoransI()

moransILigandReceptor moransILigandReceptor

# Description

Moran's I for the ligand receptor pairs

#### Usage

moransILigandReceptor

#### Format

A data frame showing the spatial autocorrelation of the 28 ligand receptor pairs

A data frame with rownames giving the 28 ligand-receptor pairs and columns moransI and pValues

#### Source

Computed using the function runMoransI on the object edgeSeurat and neighbours edgeNeighbours = computeEdgeGraph(delaunayNeighbours) with 100 trials. For more informations see the CatsCradleSpatial vignette.

mouseLRN mouseLRN

# Description

A data frame giving 11592 mouse ligand receptor pairs

# Usage

mouseLRN

# Format

a data frame with two columns, 'from' and 'to'

A data frame with two columns, 'from' and 'to'. Each row represents a mouse ligand - receptor pair.

# Source

This is taken from the nichenetr package, url = https://www.nature.com/articles/s41592-019-0667-5. Specifically, we use the mouse ligand - receptor network.

nbhdsAsEdgesToNbhdsAsList

nbhdsAsEdgesToNbhdsAsList

# Description

This function takes a set of neighbourhoods given by edges and turns it into a named list giving the memberships of each neighbourhood

# Usage

nbhdsAsEdgesToNbhdsAsList(cells, neighbourhoods)

#### Arguments

cells	• The cells whose neighbourhoods to extract.
neighbourhoods	• neighbourhoods given as a data frame with columns nodeA and nodeB, for example the output of collapseNeighbourhoods

#### Value

a named list with memberships of the neighbourhoods of cells

#### Examples

```
delaunayNeighbours = make.getExample()('delaunayNeighbours')
cells = unique(c(delaunayNeighbours[,'nodeA'],delaunayNeighbours[,'nodeB']))
nbhdsList = nbhdsAsEdgesToNbhdsAsList(cells,delaunayNeighbours)
```

neighbourhoodDiameter neighbourhoodDiameter

# Description

This function takes a list of neighbourhoods and and the centroids of the cells and finds their diameters, i.e., for each neighbourhood, the maximum distance between.

#### Usage

neighbourhoodDiameter(neighbourhoods, centroids)

#### Arguments

neighbourhoods	• a list of neighbourhoods as returned by nbhdsAsEdgesToNbhdsAsList
centroids	• the centroids of the cells

#### Value

a named numeric. The names are the names of the list neighbourhoods and the values are the maximum distance within each neighbourhood

# Examples

```
getExample = make.getExample()
centroids = getExample('centroids')
delaunayNeighbours = getExample('delaunayNeighbours')
cells = unique(c(delaunayNeighbours[,'nodeA'],delaunayNeighbours[,'nodeB']))
nbhds = nbhdsAsEdgesToNbhdsAsList(cells,delaunayNeighbours)
diameters = neighbourhoodDiameter(nbhds[seq_len(100)],centroids)
```

orderGeneSetPValues

This orders the gene set p-values (or -log10 p-values) and applies a cutoff (if given) to show only the significant gene sets for each gene cluster

# Description

This orders the gene set p-values (or -log10 p-values) and applies a cutoff (if given) to show only the significant gene sets for each gene cluster

#### Usage

```
orderGeneSetPValues(M, ascending = TRUE, cutoff = NULL, nameTag = "")
```

# Arguments

М	• A matrix of gene set p-values (or their logs) to be ordered by their significance
ascending	• Direction in which to order the columns. Defaults to TRUE, so that p- values will be ordered according to decreasing significance, should be set to FALSE if ordering -log p-value
cutoff	• if non-null this is used to extract only significant cases
nameTag	• can be used to modify the names of the list.

# Value

This returns a list of whose entries are data frames, one for each gene cluster, each giving the significant gene sets for that cluster and their significance.

performLigandReceptorAnalysis

Given a seurat object, a spatial graph, clusters and species this function identifies ligand-receptor interactions between neighbouring cells, identifies ligand-receptor interactions within and between clusters and calculates whether these are observed more frequently than expected by chance.

# Description

Given a seurat object, a spatial graph, clusters and species this function identifies ligand-receptor interactions between neighbouring cells, identifies ligand-receptor interactions within and between clusters and calculates whether these are observed more frequently than expected by chance.

# Usage

```
performLigandReceptorAnalysis(
    obj,
    spatialGraph,
    species,
    clusters,
    nSim = 1000,
    lrn = getLigandReceptorNetwork(species),
    verbose = TRUE
)
```

#### Arguments

obj	• a Seurat object
spatialGraph	• a data frame of neighbouring cell pairs.
species	• either 'human' or 'mouse'
clusters	• named vector of clusters where names are each cell and clusters are a factor
nSim	• number of simulations to perform for p value calculation.
lrn	• a ligand-receptor network, i.e., a data frame with columns from and to. By default, it retrieves the nichenetr ligand receptor network
verbose	• whether to print trace, defaults to TRUE

#### Value

A list containing: interactionsOnEdges - a data frame whose first two columns give the neighbouring cells and next two columns give their corresponding clusters. Each of the remaining columns is a logical corresponding to a ligand-receptor pair telling whether the ligand is expressed in the first cell and the receptor is expressed in the second cell. totalInteractionsByCluster - a dataframe where the first column gives a directed (sender-receiver) pair of clusters. The second column gives the total number of edges between those clusters. The remaining columns give the total numbers of edges on which particular ligand receptor interactions are present. meanInteractionsByCluster - a dataframe where the first column gives a directed (sender-receiver) pair of clusters. The second column gives the total number of edges between those clusters. The remaining columns give the total numbers of edges on which particular ligand receptor interactions are present (for that cluster pair) divided by the total number of edges between those clusters. simResults - a dataframe where the rownames are sender-receiver cluster pairs and column names are ligand receptor pairs. Values give the number of simulations for which observed values are greater than simulated values. pValues - a dataframe where the rownames are sender-receiver cluster pairs and column names are ligand receptor pairs. Entries are uppertail pvalues describing whether a particular ligand receptor interaction is observed more frequently between 2 clusters than expected.

# Examples

```
getExample = make.getExample()
smallXenium = getExample('smallXenium')
delaunayNeighbours = getExample('delaunayNeighbours')
clusters = getExample('clusters')
```

permuteMatrix This function permutes the rows of a matrix.

# Description

This function permutes the rows of a matrix.

#### Usage

permuteMatrix(M)

#### Arguments

М

a binarised expression matrix where rows are genes and columns

#### Value

This returns a matrix in which the values have been permuted within rows.

predictAnnotation	This function makes annotation predictions for a set of genes based on gene sets (e.g., hallmark) and a CatsCradle object by considering the annotations of its neighboring genes.

# Description

This function makes annotation predictions for a set of genes based on gene sets (e.g., hallmark) and a CatsCradle object by considering the annotations of its neighboring genes.

# Usage

```
predictAnnotation(
  genes,
  geneSets,
  fPrime,
  radius,
  metric = "umap",
  numPCs = NULL,
  normaliseByGeneSet = TRUE,
  normaliseByDistance = TRUE,
  normaliseToUnitVector = TRUE
)
```

#### Arguments

genes	• a character vector of genes	
geneSets	• a set of annotations, e.g., hallmark or GO	
fPrime	• a Seurat object of genes SingleCellExperiment to be converted to a Seurat object	
radius	<ul> <li>radius for prediction neighborhood</li> </ul>	
metric	• reduction or NN, defaults to umap	
numPCs	• used only if reduction is pca, defaults to NULL	
normaliseByGeneSet		
	• determines whether vector annotations are normalised by gene set size. Defaults to TRUE	
normaliseByDistance		
	• determines whether neighbor contributions are normalised by edge weight. Defaults to TRUE.	
normaliseToUnitVector		
	• determines whether to normalise returned values to unit length. Defaults to TRUE	

# Value

This returns a list of prediction vectors, one vector for each gene in genes, each vector corresponding to the sets in geneSets

#### Examples

```
getExample = make.getExample()
STranspose = getExample('STranspose',toy=TRUE)
STranspose_sce = getExample('STranspose_sce',toy=TRUE)
hallmark = getExample('hallmark',toy=TRUE)
set.seed(100)
genes = sample(colnames(STranspose),5)
predictions = predictAnnotation(genes,hallmark,STranspose,radius=.5)
predictions_sce = predictAnnotation(genes,hallmark,STranspose_sce,radius=.5)
```

predictAnnotationAllGenes

This function predicts the functions of all genes based on the functions of their neighbours.

# Description

This function predicts the functions of all genes based on the functions of their neighbours.

# Usage

```
predictAnnotationAllGenes(
  geneSets,
  fPrime,
  radius,
  metric = "umap",
  normaliseByGeneSet = TRUE,
  normaliseByDistance = TRUE,
  normaliseToUnitVector = TRUE
)
```

# Arguments

geneSets	• a set of gene sets, e.g., hallmark	
fPrime	• a transposed Seurat object (generated with transposeObject()) or Single-	
	CellExperiment to be converted to a Seurat object	
radius	<ul> <li>radius of the region to use for prediction</li> </ul>	
metric	• reduction or NN, defaults to umap	
normaliseByGeneSet		
	<ul> <li>normalise by size of each gene set, defaults to TRUE</li> </ul>	
normaliseByDistance		
	• attenutate neighbour contributions based on distance, defaults to TRUE	
normaliseToUnitVector		
	• return results as unit vectors, defaults to TRUE	

# Value

• A list where names are genes and values are vectors of gene annotations whose entries correspond to the geneSets

# Examples

```
getExample = make.getExample()
STranspose = getExample('STranspose',toy=TRUE)
hallmark = getExample('hallmark',toy=TRUE)
predictions = predictAnnotationAllGenes(hallmark,STranspose,radius=.5)
```

```
predictGeneAnnotationImpl
```

This function is the implementation for predicting the functions of a gene based on the functions of its neighbours.

# Description

This function is the implementation for predicting the functions of a gene based on the functions of its neighbours.

# randomiseGraph

# Usage

```
predictGeneAnnotationImpl(
  gene,
  fPrime,
  genesAnno,
  radius,
  metric,
  numPCs = NULL,
  normaliseByDistance = TRUE
)
```

#### Arguments

gene	• gene to annotate
fPrime	• a Seurat object of genes or SingleCellExperiment to be converted to a Seurat object
genesAnno	• genes annotated with gene sets
radius	<ul> <li>radius of neighbours to consider</li> </ul>
metric	• which metric to use to discover neighbours, can be one of 'umap', 'tsne', 'pca', 'NN', defaults to umap
numPCs	• used only if metric is pca. Defaults to NULL
normaliseByDistance	
	• choose whether to normalise contributions of neighbors by their distance, defaults to TRUE

#### Value

This returns a named list. The names are the anotations that apply to the neighbour genes, the values are the relative wieghts of the contributions.

# Examples

```
getExample = make.getExample()
STranspose = getExample('STranspose',toy=TRUE)
hallmark = getExample('hallmark',toy=TRUE)
genesAnno = annotateGenesByGeneSet(hallmark)
predictions = predictGeneAnnotationImpl('Myc',STranspose,genesAnno,
radius=.5,metric='umap')
```

randomiseGraph This function performs degree-preserving randomisation of neighbour graphs.	randomiseGraph		
--	----------------	--	--

# Description

This function performs degree-preserving randomisation of neighbour graphs.

# Usage

randomiseGraph(spatialGraph, maxTries = 1000)

# Arguments

spatialGraph	• a spatial graph in neighbour list format.
maxTries	• the maximum number of tries to remove self edges during graph randomi-
	sation. If self edges are remeining this will be reported.

# Value

A randomised graph where degree from the original graph is preserved.

randomiseNodeIndices This function generates random indices for node B

# Description

This function generates random indices for node B

#### Usage

```
randomiseNodeIndices(neighborListDf, n = 100, useWeights = FALSE)
```

# Arguments

neighborListDf	• a dataframe containing the neighborlist
n	• the number of times to randomise indices
useWeights	• whether to preserve edgeweights.

# Value

• a matrix with randomised indices for node B

# Examples

```
NN = make.getExample()('NN')
NN = desymmetriseNN(NN)
randomIndices = randomiseNodeIndices(NN,10,TRUE)
```

readGmt

# Description

This function reads in gene sets in .gmt format

#### Usage

```
readGmt(gmtFile, addDescr = FALSE)
```

# Arguments

gmtFile	• a .gmt file containing gene sets, e.g., Hallmark of GO
addDescr	• include gene set description (2nd column in .gmt file) in gene set name

# Value

• A named list of gene sets

```
runGeometricClusteringTrials
```

This runs random trials to determine the statistical significance of the clustering of a set of points within a larger set.

# Description

This function takes a matrix whose rows are geometric coordinates and a subset of these points either given as a character vector which is a subset of the rownames or as a logical vector. It returns statistics on the mean distance of the complement to the subset.

# Usage

```
runGeometricClusteringTrials(S, geneSubset, numTrials)
```

# Arguments

S	• a set of points given as a matrix. The rows are the coordinates of these points
geneSubset	- this is either a subset of the rownames of $S$ or a logical whose length is $\ensuremath{nrow}(S)$
numTrials	• the number or random trials to perform

# Value

This returns a list. subsetDistance gives the median complement distance for the actual set, randomSubsetDistance gives the complement distances for the numTrials random sets, pValue gives a p-value based on the rank of the actual distance among the random distances and zScore gives its z-score.

# Examples

```
library(Seurat)
getExample = make.getExample()
STranspose = getExample('STranspose',toy=TRUE)
hallmark = getExample('hallmark')
S = data.matrix(FetchData(STranspose,c('umap_1','umap_2')))
geneSubset = rownames(S) %in% hallmark[["HALLMARK_TNFA_SIGNALING_VIA_NFKB"]]
geneClustering = runGeometricClusteringTrials(S,geneSubset,100)
```

runMoransI	This function takes a matrix where rows are features and columns are
	cells, and a neighbourhood list, and computes Moran's I.

#### Description

This function takes a matrix where rows are features and columns are cells, and a neighbourhood list, and computes Moran's I.

# Usage

```
runMoransI(
   obj,
   spatialGraph,
   assay = "RNA",
   layer = "data",
   nSim = 100,
   verbose = TRUE
)
```

#### Arguments

obj	• a Seurat object
spatialGraph	• a data frame of neighbouring cell pairs.
assay	• assay to pull data from, defaults to RNA.
layer	• layer to pull data from, defaults to data.
nSim	• number of simulations to perform for p value calculation. Defaults to 100.
verbose	• whether to print trace, defaults to TRUE

# sankeyFromMatrix

# Value

a dataframe containing Moran's I and p values for each feature.

# Examples

```
getExample = make.getExample()
smallXenium = getExample('smallXenium',toy=TRUE)
delaunayNeighbours = getExample('delaunayNeighbours',toy=TRUE)
moransI = runMoransI(smallXenium, delaunayNeighbours, assay = "SCT",
layer = "data", nSim = 10, verbose = FALSE)
```

sankeyFromMatrix	This makes a sankey graph from a matrix of average expression. Our
	"Cat's Cradle".

# Description

This makes a sankey graph from a matrix of average expression. Our "Cat's Cradle".

#### Usage

```
sankeyFromMatrix(
    M,
    disambiguation = c("R_", "C_"),
    fontSize = 20,
    minus = "red",
    plus = "blue",
    height = 1200,
    width = 900
)
```

# Arguments

Μ	• a matrix of gene expression
disambiguation	• used to distinguish between the row names and the column names if these overlap
fontSize	• defaults to 20
minus	• colour to use for links with negative values
plus	• colour for positive values
height	• height in pixels, defaults to 1200
width	• width in pixels, defaults to 900

# Value

A sankey graph

# Examples

```
set.seed(100)
M = matrix(runif(12)-.3,nrow=3)
rownames(M) = as.character(seq_len(3))
colnames(M) = as.character(seq_len(4))
sankey = sankeyFromMatrix(M)
```

seuratCells seuratCells

### Description

A vector of cells used for subsetting exSeuratObj

# Usage

seuratCells

# Format

A vector of cells

A vector of cells consisting of half the cells from each seurat\_cluster in exSeuratObj used to subset this object to give toy examples.

#### Source

Computed by retrieving half the cells from each cluster in exSeuratObj

seuratGenes	seuratGenes		
-------------	-------------	--	--

# Description

A vector of genes used for subsetting exSeuratObj

#### Usage

seuratGenes

#### Format

A vector of genes

A vector of the top 100 most variable genes in exSeuratObj used to subset this object to give toy examples.

#### Source

Computed by retrieving the data layer from exSeuratObj and subsetting to the 100 genes with the highest standard deviation.

smallXenium

smallXenium

#### Description

A spatial Seurat object of 4261 cells and 248 genes

# Usage

smallXenium

# Format

A Seurat object

A spatial Seurat object subset from the Xenium object used in https://satijalab.org/seurat/articles/seurat5\_spatial\_vignett

#### Source

This is subset from the Xenium spatial Seurat object https://cf.10xgenomics.com/samples/xenium/1.0.2/Xenium\_V1\_FF\_Mo to include a small region of the field of view surrounding the dentate gyrus.

stripGeneSet	This function strips out non-gene information from the beginning of
	GO sets, etc.

# Description

This function strips out non-gene information from the beginning of GO sets, etc.

# Usage

```
stripGeneSet(geneSet)
```

# Arguments

geneSet • a list of gene sets

# Value

a named list of gene sets

symmetriseNN

#### Description

This first checks to see if the NN graph is symmetric and if not symmetrises it.

# Usage

symmetriseNN(NN)

# Arguments

NN

• a nearest neighbors graph as returned by getNearestNeighbourLists

#### Value

a nearest neighbors graph

#### Examples

```
NN = make.getExample()('NN',toy=TRUE)
NNStar = symmetriseNN(NN)
```

symmetryCheckNN Tests whether a nearest neighbor graph is symmetric

# Description

The nearest neighbor relationship is not inherently symmetric. This tests whether the nearest neighbor graph retrieved from a Seurat object is.

# Usage

symmetryCheckNN(NN)

#### Arguments

NN

• a nearest neighbor graph. This is in the form of a data frame as returned by getNearestNeighbourLists. Its coloumns include nodeA and nodeB.

# Value

TRUE or FALSE

#### Examples

```
NN = make.getExample()('NN',toy=TRUE)
symmetryTest = symmetryCheckNN(NN)
```

tagRowAndColNames This gussies up the rownames and colnames of M

# Description

This gussies up the rownames and colnames of M

# Usage

```
tagRowAndColNames(M, ccTag = "CC_", gcTag = "GC_")
```

# Arguments

М	• a matrix, typically the average expression matrix
ccTag	• a prefix for the row (cell cluster) names
gcTag	• a prefix for the column (gene cluster) names

# Value

The same matrix with fancier row and col names

# Examples

```
getExample = make.getExample()
averageExpMatrix = getExample('averageExpMatrix',toy=TRUE)
averageExpMatrix = tagRowAndColNames(averageExpMatrix,'cellCluster_','geneCluster_')
```

transposeObject Create the transpose of a Seurat object

# Description

This takes a Seurat object f and creates a new Seurat object whose expression matrix is the transpose of that of f. This can also be a SingleCellExperiment which will be converted to a Seurat object

# Usage

```
transposeObject(
  f,
  active.assay = "RNA",
  npcs = 30,
  dims = seq_len(20),
  res = 1,
  returnType = "Seurat",
  verbose = FALSE
)
```

# Arguments

f	• a Seurat object
active.assay	• the assay to use. Defaults to 'RNA'
npcs	• number of principal components, defaults to 30
dims	• dimensions to use for umap and nearest neighbors, defaults to 1:20
res	• the clustering resolution, defaults to 1
returnType	• Will return a SingleCellExperiment if this is either of SCE, SingleCellExperiment or their lower-case equivalents. Otherwise, returns a Seurat object
verbose	• Controls whether to display trace from the Seurat functions. Defaults to FALSE

# Value

A Seurat object or SingleCellExperiment

# Examples

```
exSeuratObj = make.getExample()('exSeuratObj',toy=TRUE)
STranspose = transposeObject(exSeuratObj)
STransposeAsSCE = transposeObject(exSeuratObj,returnType='SCE')
```

xeniumCells xeniumCells

# Description

A vector of cells used for subsetting exSeuratObj

# Usage

xeniumCells

# Format

A vector of cells

A vector of cells consisting of approximately one quarter of the cells in smallXenium used to subset this object to give toy examples.

# Source

We extracted a rectangle whose width and height were one half the width and height of smallXenium and which was centered in the field of view of smallXenium

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