crossWGCNA R package

September 29, 2023

Description

Function to compute cross-tissue gene expression adjacencies.

Usage

```
Adjacency(data, method="selfloop",comp1="_tis1",comp2="_tis2", Adj_type="signed", cortype="spearman", pval="none", thr=0.05, beta=6, verbose=FALSE)
```

data	Input data matrix, where rows are genes and columns are samples. Gene expression measures in the two tissues are combined by row.
method	String character identifying the method to use to compute cross-tissue adjacencies. Can be "self-loop" or "netdiff".
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".
Adj_type	String character identifying the type of adjacency to compute. Can be "signed", "unsigned" or "keep sign". Default="signed". "keep sign" is equivalent to "unsigned" in absolute values, but with sign corresponding to the sign of correlation. See WGCNA package for details.
cortype	String character identifying the type of correlation. Can be "pearson", "spearman" or "bicor". Default="spearman". See WGCNA package for details.
pval	String character identifying whether to use the correlations' p-value in edge weight. Possible values are: "none" (does not include p-values), "threshold" (discard edges with a p-value higher than a threshold), "weight" (multiply edges' adjacencies by 1-pvalue). Default="none". Can be used with "pearson" and "spearman" correlations.

thr Threshold for cutting edges based on p-values. Default=0.05.

beta Power for adjacency calculation. Default=6.

verbose Boolean value. If TRUE prints logging output. Default=FALSE.

Details

The adjacencies are computed accordingly to the method selected (i.e., "self-loop" or "netdiff").

Value

Returns a matrix with pair-wise gene adjacencies.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

References

Langfelder P, Horvath S (2008). WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics, 559.

See Also

```
rm_selfloop, rm_netdiff
```

Examples

See package vignette.

```
ST_expr_smooth_output
```

 $Averaged\ gene\ expression\ of\ {\tt BC@assays\$SCT@data}$

Description

Pre-computed smooths spatial transcriptomics gene expression profiles using the weighted mean of neighbouring spots in one compartment. Data were derived from a Breast Cancer Visium dataset provided by the 10x genomics.

Usage

```
load("ST_expr_smooth_out.RData")
```

Format

A data frame 20227 genes x 4898 spots.

Details

Output of ST_expr_smooth function. The object is available for download at zenodo web page: codehttps://zenodo.org/record/8268805.

See Also

```
ST_expr_smooth
```

Examples

```
options(timeout=200)
download.file(
  url="https://zenodo.org/record/8268805/files/ST_expr_smooth_out.RData?download=1",
  destfile="~/ST_expr_smooth_out.RData")
load("~/ST_expr_smooth_out.RData")
```

```
Breast Cancer ST Seurat Object

ST Seurat Object
```

Description

Pre-computed clusters derived from a Breast Cancer Visium dataset provided by the 10x genomics.

Details

A common Seurat (version 4.1.3) analysis pipeline has been applied on the "h5" file to finally obtain clusters and umaps. See Seurat package for details. The object is available for download at zenodo web page: codehttps://zenodo.org/record/8268805.

Examples

clusteringWGCNA

clusteringWGCNA

Description

Function to perform WGCNA clustering based on an adjacency matrix.

Usage

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Arguments

A	Adjacency matrix. Should be the output of the function Adjacency. Must be a symmetric matrix.
data	Input data matrix, where rows are genes and columns are samples. Gene expression measures in the two tissues are combined by row.
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".
TOM	Boolean value. If TRUE compute the Topological Overlap Matrix prior to clustering. Default=TRUE.
ds	Parameter influencing the final number of clusters. It sets the deepSplit parameter in the cutreeDynamic function from the WGCNA package.
crossOnly	Boolean value. If TRUE remove intra-tissue adjacencies prior to clustering (set to 0). Default=TRUE.

Value

Returns a list of connectivities (output of degrees) and of genes' clusters.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

References

Langfelder P, Horvath S (2008). WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics, 559.

See Also

```
Adjacency, rm_selfloop, degrees, degrees_mod.
```

Examples

```
See package vignette.
```

Description

Plot the correlation between two genes in the same or in different compartments/tissues.

Usage

```
cor_inspect(data, gene1, gene2, comp1="_tis1", comp2="_tis2")
```

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Arguments

data	Input data matrix, where rows are genes and columns are samples. Gene expression measures in the two tissues are combined by row.
gene1	First gene to plot
gene2	Second gene to plot
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".

Value

A plot with four panels displaying the scatterplots of gene1 and gene2 relationships within and between tissues/compartments.

Author(s)

Aurora Savino, Raffaele M. Iannzzi.

Examples

See package vignette.

|--|

Description

One-step function to obtain connectivities of each gene with all genes of the same or of the alternate tissue/organ, and gene clusters based on inter-tissue connectivities.

Usage

data	Input data matrix, where rows are genes and columns are samples. Gene expression measures in the two tissues are combined by row.
method	String character identifying the method to use to compute cross-tissue adjacencies. Can be "self-loop" or "netdiff".
Adj_type	String character identifying the type of adjacency to compute. Can be "signed", "unsigned" or "keep sign". Default="signed". "keep sign" is equivalent to "unsigned" in absolute values, but with sign corresponding to the sign of correlation. See WGCNA package for details.

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cortype	String character identifying the type of correlation. Can be "pearson", "spearman" or "bicor". Default="spearman".
pval	String character identifying whether to use the correlations' p-value in edge weight. Possible values are: "none" (does not include p-values), "threshold" (discard edges with a p-value higher than a threshold), "weight" (multiply edges' adjacencies by 1-pvalue). Default="none". Can be used with "pearson" and "spearman" correlations.
thr	Threshold for cutting edges based on p-values. Default=0.05.
beta	Power for adjacency calculation. Default=6.
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".
doClusters	Boolean value. If TRUE compute clusters. Default=TRUE.
doTOM	Boolean value. If TRUE compute the Topological Overlap Matrix prior to clustering. Default=TRUE.
ds	Parameter influencing the final number of clusters. It sets the deepSplit parameter in the cutreeDynamic function from the WGCNA package.
crossOnly	Boolean value. If TRUE remove intra-tissue adjacencies prior to clustering (set to 0). Default=TRUE.
verbose	Boolean value. If TRUE prints logging output. Default=TRUE.

Value

If doClusters is set to FALSE, returns a list of connectivities otherwise returns both list of connectivities and gene modules.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

References

Langfelder P, Horvath S (2008). WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics, 559.

See Also

Adjacency, rm_selfloop, rm_netdiff, degrees, clusteringWGCNA.

Examples

See package vignette.

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cytoscape_net cytoscape_net

Description

Finds the top cross-tissue interactors of a gene and saves them as a Cytoscape input.

Usage

```
cytoscape_net(A, data, gene, comp1="_tis1", comp2="_tis2", num, corr="spearman")
```

Arguments

A	Adjacency matrix. Should be the output of the function Adjacency. Must be a symmetric matrix.
data	Input data matrix, where rows are genes and columns are samples. Gene expression measures in the two tissues are combined by row.
gene	Gene to be analyzed (string character).
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".
num	Number (numeric) of first closest neigbours of the selected genes to consider.
corr	String character identifying the type of correlation. Can be "pearson", "spearman" or "bicor". Default="spearman".

Value

Dataframe with the top interactors of the selected gene and their pairwise adjacencies within and between tissues. Columns of the dataframe are:

Source	source gene
Target	connected gene
Weight	connection strenght: adjacency
Edge_type	type of interaction: within compartment 1 (intra1), compartment 2 (intra2) or between compartments (inter)
Source_type	label indicating whether the source gene has been measured in compartment $\boldsymbol{1}$ or compartment $\boldsymbol{2}$
Target_type	label indicating whether the target gene has been measured in compartment 1 or compartment 2

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

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References

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Research 2003 Nov; 13(11):2498-504

Examples

See package vignette.

Usage

data	Input data matrix, where rows are genes and columns are samples. Gene expression measures in the two tissues are combined by row.
method	String character identifying the method to use to compute cross-tissue adjacencies. Can be "self-loop" or "netdiff".
modules	List of co-expression modules. Output of ${\tt clusteringWGCNA}$
Adj_type	String character identifying the type of adjacency to compute. Can be "signed", "unsigned" or "keep sign". Default="signed". "keep sign" is equivalent to "unsigned" in absolute values, but with sign corresponding to the sign of correlation. See WGCNA for details.
cortype	String character identifying the type of correlation. Can be "pearson", "spearman" or "bicor". Default="spearman". See WGCNA for details.
pval	String character identifying whether to use the correlations' p-value in edge weight. Possible values are: "none" (does not include p-values), "threshold" (discard edges with a p-value higher than a threshold), "weight" (multiply edges' adjacencies by 1-pvalue). Default="none". Can be used with "pearson" and "spearman" correlations.
thr	Threshold for cutting edges based on p-values. Default=0.05.
beta	Power for adjacency calculation. Default=6.
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".

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Value

For each module returns a list of degrees' vectors:

kInt1	Intra-tissue degree for tissue 1
kInt2	Intra-tissue degree for tissue 2
kExt1	Inter-tissue degree for tissue 1 genes with tissue 2
kExt2	Inter-tissue degree for tissue 2 genes with tissue 1
kTot1	Overall connectivity of genes in tissue 1 with genes in both tissue 1 and 2
kTot2	Overall connectivity of genes in tissue 2 with genes in both tissue 1 and 2

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

References

Langfelder P, Horvath S (2008). WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics, 559.

See Also

```
degrees, Adjacency, clustering WGCNA, cross WGCNA
```

Examples

See package vignette.

Description

Function to compute the weighted degree of a gene with all genes of the same or of the alternate tissue/organ.

Usage

```
degrees(A, comp1="_tis1", comp2="_tis2")
```

A	Matrix of adjacencies, output of the function Adjacency.
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".

Value

A list of degrees' vectors:

kInt1	Intra-tissue degree for tissue 1
kInt2	Intra-tissue degree for tissue 2
kExt1	Inter-tissue degree for tissue 1 genes with tissue 2
kExt2	Inter-tissue degree for tissue 2 genes with tissue 1
kTot1	Overall connectivity of genes in tissue 1 with genes in both tissue 1 and 2
kTot2	Overall connectivity of genes in tissue 2 with genes in both tissue 1 and 2

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

References

Langfelder P, Horvath S (2008). WGCNA: an R package for weighted correlation network analysis.Returns a list of connectivities BMC Bioinformatics, 559.

See Also

Adjacency, clustering WGCNA, cross WGCNA, degrees_mod.

Examples

```
See package vignette.
```

```
"epi" GSE5847 RData Object

Epithelium transcriptome profile.
```

Description

Laser Capture Microdissection (LCM) reduced toy dataset with matched stroma transcriptome profiles from the same tumor (GSE5847).

Usage

```
load("epi_GSE5847.RData")
```

Format

A data frame with 3101 genes \times 34 samples.

Details

The dataset is automatically loaded with crossWGCNA library loading.

Source

```
codehttps://www.mdpi.com/2072-6694/13/13/3371
```

References

Savino, A., de Marzo, N., Provero, P. & Poli, V. Meta-analysis of microdissected breast tumors reveals genes regulated in the stroma but hidden in bulk analysis. Cancers (Basel) 13, (2021).

Examples

```
load("epi_GSE5847.RData")
```

```
"stroma" GSE5847 RData Object
```

Stroma transcriptome profile.

Description

Laser Capture Microdissection (LCM) reduced toy dataset with matched epithelium transcriptome profiles from the same tumor (GSE5847).

Usage

```
load("stroma_GSE5847.RData")
```

Format

A data frame with 3101 genes \times 34 samples.

Details

The dataset is automatically loaded with crossWGCNA library loading.

Source

```
codehttps://www.mdpi.com/2072-6694/13/13/3371
```

References

Savino, A., de Marzo, N., Provero, P. & Poli, V. Meta-analysis of microdissected breast tumors reveals genes regulated in the stroma but hidden in bulk analysis. Cancers (Basel) 13, (2021).

```
load("stroma_GSE5847.RData")
```

rm_netdiff

Description

Removes the average connectivity between each gene pair from both tissues<e2><80><99> networks.

Usage

```
rm_netdiff(A, comp1="_tis1", comp2="_tis2", verbose=TRUE)
```

Arguments

A	Matrix of adjacencies, output of the function Adjacency.
comp1	Suffix (string character) to identify genes of tissue 1. Default=" $_$ tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".
verbose	Boolean value. If TRUE prints logging output. Default=FALSE.

Details

See the manuscript for further details.

Value

Returns the adjusted Adjacency matrix.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

See Also

```
rm_netdiff
```

rm_selfloop 13

Description

Function that imposes self-loops to a zero value.

Usage

```
rm_selfloop(A, comp1="_tis1",comp2="_tis2", verbose=TRUE)
```

Arguments

A	Matrix of adjacencies, output of the function Adjacency.
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".
verbose	Boolean value. If TRUE prints logging output. Default=FALSE.

Details

See the manuscript for further details.

Value

Returns the adjusted Adjacency matrix.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

See Also

```
rm_netdiff
```

```
ST_boundary_spots ST_boundary_spots
```

Description

Generates matched vectors of indexes for compartment 1 and 2 spots for spatial transcriptomics data.

Usage

```
ST_boundary_spots(included_spots, coords)
```

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Arguments

included_spots

Spots of tissue compartment 1 along the boundaries of compartment 1 and 2.

coords X and y coordinates for each spot, obtained from the ST_spots_coords

function.

Value

List of two vectors of indexes (matched) for compartment 1 and compartment 2 spots. Wherever the same spot is neighbouring multiple spots of the alternate compartment, the spot index is repeated to represent both.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

See Also

```
ST_expr_smooth, ST_merged_dataset, ST_midpoints_def, ST_plot_comm, ST_plot_expr, ST_spots_coords, ST_spots_filt, ST_weighted_mod.
```

Examples

See package vignette.

```
ST_expr_smooth
```

Description

Smooths gene expression using the weighted mean of neighbouring spots in the same tissue compartment for spatial transcriptomics data.

Usage

```
ST_expr_smooth(expr_data, coords, max_dist, spots_class, sel_class)
```

expr_data	Input data matrix, where rows are genes and columns are spots.
coords	Dataframe with columns specyfing x and y coordinates for each spot, obtained from the ST_spots_coords function.
max_dist	Integer specifying maximum spots distance to be included in the weighed mean. Default=5.
spots_class	Charater vector with labels indicating the compartment to which each spot belongs.
sel_class	Character vector with the labels of the two compartments to analyze. Can be "Epi" and "Stroma".

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Details

Input data is obtained from @assay\$SCT@data slot of the Seurat object.

Value

Smoothed data matrix, where rows are genes and columns are spots.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

See Also

```
ST_boundary_spots, ST_merged_dataset, ST_midpoints_def, ST_plot_comm, ST_plot_expr, ST_spots_coords, ST_spots_filt, ST_weighted_mod.
```

Examples

```
See package vignette.
```

```
ST_merged_dataset ST_merged_dataset
```

Description

Creates a merged dataset with gene expression from the two selected tissue compartments for spatial transcriptomics data.

Usage

sel_spots	List of two vectors, one for each compartment, with indexes of spots to be included. Output of the ST_spots_filt function.
coords	X and y coordinates for each spot, obtained from the ST_spots_coords function.
averaged_expr_all	
	Gene expression data matrix where rows are genes and columns are spots. Out-
	<pre>put of the ST_expr_smoothfunction.</pre>
var_thr	Threshold (numeric) with the lowest gene expression variance percentile to keep a gene in the final data matrix. Default=0.75.
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".

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Details

For each selected spot in compartment 1, takes the neighbouring spots in compartment 2 and creates a gene expression matrix with matched gene expression from the two selected tissue compartments.

Value

List of two values:

data_merged Matrix with gene expression from the two selected compartments, where columns represent positions in space (of selected compartment 1 spots), and rows are genes measured either in one or in the other compartment (denoted by different suffixes).

included_spots

Vector of indexes for the compartment 1 spots included in the final data matrix.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi

See Also

```
ST_expr_smooth, ST_merged_dataset, ST_midpoints_def, ST_plot_comm, ST_plot_expr, ST_spots_coords, ST_spots_filt, ST_boundary_spots, ST_weighted_mod
```

Examples

See package vignette.

Description

Defines boundaries between two tissues compartments as the points equally distant from two neighbouring spots for spatial transcriptomics data.

Usage

```
ST_midpoints_def(coords, sel_spots)
```

coords	X and y coordinates for each spot, obtained from the ST_spots_coords function.
sel_spots	List of two vectors of indexes (matched) for compartment 1 and compartment 2
	spots. Output of the ST_boundary_spots function.

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Value

List of two vectors with x and y coordinates of the midpoints.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

See Also

```
ST_expr_smooth, ST_merged_dataset, ST_plot_comm, ST_plot_expr, ST_spots_coords,
ST_spots_filt, ST_boundary_spots, ST_weighted_mod
```

Examples

See package vignette.

ST_plot_comm

ST_plot_comm

Description

Generates a plot showing the communication score of two genes along the boundries between the two tissue compartments for spatial transcriptomics data.

Usage

```
ST_plot_comm(gene1, gene2, averaged_expr_all, coords, included_spots,
             sel_spots, tis1_spots, tis2_spots, midpoints)
```

Arguments

gene1	First gene, measured in compartment 1.		
gene2	Second gene, measured in compartment 2.		
averaged_expr_all			
	Gene expression data matrix where rows are genes and columns are spots. Output of the ST_expr_smooth function.		
coords	X and y coordinates for each spot, obtained from the ST_spots_coords function.		
included_spots			
	Spots of compartment 1 along the boundaries of compartment 1 and 2.		
sel_spots	List of two vectors of indexes (matched) for compartment 1 and compartment 2 spots. Output of the ST_boundary_spots function.		
tis1_spots	Indexes of compartment 1 spots.		
tis2_spots	Indexes of compartment 2 spots.		
midpoints	List of two vectors with x and y coordinates of the midpoints between neighbouring compartment 1 and compartment 2 spots. Output of the $ST_midpoints_df$ function.		

ST_plot_expr

Value

Returns a plot.

Author(s)

Aurosa Savino, Raffaele M. Iannuzzi.

See Also

```
ST_expr_smooth, ST_merged_dataset, ST_midpoints_def, ST_plot_expr, ST_spots_coords, ST_spots_filt, ST_boundary_spots, ST_weighted_mod.
```

Examples

See package vignette.

ST_plot_expr

Plot gene expression in space

Description

Generates a plot showing the expression of a gene in space and the boundaries of the two tissue compartments for spatial transcriptomics data.

Usage

gene	Gene to be plotted.		
averaged_expr_all			
	Gene expression data matrix where rows are genes and columns are spots. Output of the ST_expr_smooth function.		
coords	X and y coordinates for each spot, obtained from the ${\tt ST_spots_coords}$ function.		
included_spots			
	Spots of compartment 1 along the boundaries of compartment 1 and 2.		
tis1_spots	Vector of indexes of spots belonging to tissue 1		
tis2_spots	Vector of indexes of spots belonging to tissue 2		
midpoints	List of two vectors with x and y coordinates of the midpoints between neighbouring compartment 1 and compartment 2 spots. Output of the $ST_midpoints_def$ function.		

ST_spots_coords 19

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

See Also

```
ST_expr_smooth, ST_merged_dataset, ST_midpoints_def, ST_plot_comm, ST_spots_coords, ST_spots_filt, ST_boundary_spots, ST_weighted_mod.
```

Examples

```
See package vignette.
```

```
ST_spots_coords
```

ST_spots_coords

Description

Defines the x and y coordinates of each spot in the array for spatial transcriptomics data.

Usage

```
ST_spots_coords (data, br=1000)
```

Arguments

data Seurat object with the spatial transcriptomics data.

Number of breaks (numeric) for finding the coordinate peaks. Needs to be higher

than the number of actual x or y coordinates of spots in the array. Default=1000

Details

Assign the value of 1 to the leftmost spot considering an inter-spot x distance of 1

Value

Returns a matrix with two columns for x and y coordinates of each spot.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

See Also

```
ST_expr_smooth, ST_merged_dataset, ST_midpoints_def, ST_plot_expr, ST_plot_comm ST_spots_filt, ST_boundary_spots, ST_weighted_mod.
```

```
See package vignette.
```

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|--|--|

Description

Selects spots with at least one neighbouring spot belonging to the same tissue compartment for spatial transcriptomics data.

Usage

```
ST_spots_filt(coords, tis1_spots, tis2_spots)
```

Arguments

coords	\boldsymbol{X} and y coordinates for each spot, obtained from the ST_spots_coords function.
tis1_spots	Vector of indexes of spots belonging to tissue 1
tis2_spots	Vector of indexes of spots belonging to tissue 2

Value

List of two vectors: indexes of selected spots for tissue 1; indexes of selected spots for tissue 2.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

See Also

```
ST_expr_smooth, ST_merged_dataset, ST_midpoints_def, ST_plot_expr, ST_plot_comm, ST_spots_coords, ST_spots_filt, ST_boundary_spots, ST_weighted_mod.
```

```
See package vignette.
```

ST_weighted_mod 21

Description

Computes a weighted summmary of a module's expression for spatial transcriptomics data.

Usage

Arguments

modules	Vector with module labels for each gene.	
kw	Connectivities computed with the function kwithin.	
mod_sel	Label of the selected module.	
averaged_expr_all		
	Gene expression data matrix where rows are genes and columns are spots. Output of the ST_expr_smooth function.	
comp1	Suffix (string character) to identify genes of tissue 1. Default="_tis1".	
comp2	Suffix (string character) to identify genes of tissue 2. Default="_tis2".	

Details

The computation is based on the kExt of the genes measured in each compartment separately.

Value

List of two vectors with the weighted mean of the expression of module's genes belonging to compartment 1 or compartment 2.

Author(s)

Aurora Savino, Raffaele M. Iannuzzi.

See Also

```
ST_expr_smooth, ST_merged_dataset, ST_midpoints_def, ST_plot_expr, ST_plot_comm, ST_spots_coords, ST_spots_filt, ST_boundary_spots.
```

```
See package vignette.
```

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