

# Package ‘RegionalST’

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**Type** Package

**Title** Investigating regions of interest and performing regional cell type-specific analysis with spatial transcriptomics data

**Version** 1.4.2

**Description** This package analyze spatial transcriptomics data through cross-regional cell type-specific analysis. It selects regions of interest (ROIs) and identifies cross-regional cell type-specific differential signals. The ROIs can be selected using automatic algorithm or through manual selection. It facilitates manual selection of ROIs using a shiny application.

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**Depends** R (>= 4.3.0)

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**Author** Ziyi Li [aut, cre]

**Maintainer** Ziyi Li <zli16@mdanderson.org>

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---

DoGSEA

*Perform GSEA analysis for cross-regional DE genes*

---

### Description

Perform GSEA analysis for cross-regional DE genes

### Usage

```
DoGSEA(considerRes, whichDB = "hallmark", gmtdir = NULL, withProp = FALSE)
```

### Arguments

|             |                                                                                        |
|-------------|----------------------------------------------------------------------------------------|
| considerRes | A list of cross-regional DE genes.                                                     |
| whichDB     | A character string to select the database names, e.g., "hallmark", "kegg", "reactome". |
| gmtdir      | Directory for external database gmt file location.                                     |
| withProp    | Whether deconvolution proportion is used in previous steps.                            |

### Value

A list including GSEA results for all cell types.

**Examples**

```
data(exampleRes)
allCTres <- DoGSEA(exampleRes, whichDB = "hallmark", withProp = TRUE)
```

---

DrawDotplot

*Draw dot plot for GSEA results of cross-regional DE genes*


---

**Description**

Draw dot plot for GSEA results of cross-regional DE genes

**Usage**

```
DrawDotplot(
  allCTres,
  CT = 1,
  angle = 20,
  vjust = 0.9,
  hjust = 1,
  padj_cutoff = 1,
  topN = 20,
  chooseP = "padj",
  eachN = NULL
)
```

**Arguments**

|             |                                                                                   |
|-------------|-----------------------------------------------------------------------------------|
| allCTres    | A list of GSEA results for all cell types.                                        |
| CT          | A number of the interested cell type, e.g., 1, 2, 3.                              |
| angle       | A number of plotting parameter, angle of the x axis label.                        |
| vjust       | A number of vertical adjustment in plotting.                                      |
| hjust       | A number of horizontal adjustment in plotting.                                    |
| padj_cutoff | A cutoff number of adjusted p value.                                              |
| topN        | A number of the plotted top pathways.                                             |
| chooseP     | A character string for the p value that used in plotting, e.g., "padj" or "pval". |
| eachN       | The maximum number of pathways in each cell type.                                 |

**Value**

A plot object

**Examples**

```
data(exampleRes)
allCTres <- DoGSEA(exampleRes, whichDB = "hallmark", withProp = TRUE)
DrawDotplot(allCTres, CT = 1, angle = 15, vjust = 1, chooseP = "padj")
```

---

DrawRegionProportion *Draw regional cell type distribution with cell type annotation information*

---

### Description

Draw regional cell type distribution with cell type annotation information

### Usage

```
DrawRegionProportion(sce, label = "celltype", selCenter = seq_len(10))
```

### Arguments

|           |                                                |
|-----------|------------------------------------------------|
| sce       | A single cell experiment object.               |
| label     | A string character for the cell type variable. |
| selCenter | A vector of the interested ROIs, e.g., 1:4.    |

### Value

A plot object.

### Examples

```
data("example_sce")
DrawRegionProportion(example_sce, label = "celltype", selCenter = 1:3)
```

---

DrawRegionProportion\_withProp  
*Draw regional cell type distribution with cellular proportion information*

---

### Description

Draw regional cell type distribution with cellular proportion information

### Usage

```
DrawRegionProportion_withProp(
  sce,
  label = "CARD_CellType",
  selCenter = seq_len(10)
)
```

### Arguments

|           |                                                |
|-----------|------------------------------------------------|
| sce       | A single cell experiment object.               |
| label     | A string character for the cell type variable. |
| selCenter | A vector of the interested ROIs, e.g., 1:4.    |

**Value**

A plot object.

**Examples**

```
data("example_sce")
DrawRegionProportion_withProp(example_sce,
                               label = "Proportions",
                               selCenter = 1:3)
```

---

exampleRes

*Example DE output*

---

**Description**

A simulated example DE output file

**Usage**

```
data(exampleRes)
```

**Format**

A list object.

**Value**

A list object.

**Examples**

```
data(exampleRes)
```

---

example\_sce

*Example single cell experiment for input*

---

**Description**

A simulated example input data file

**Usage**

```
data(example_sce)
```

**Format**

A SingleCellExperiment object.

**Value**

A SingleCellExperiment object.

**Examples**

```
data(example_sce)
```

---

FindRegionalCells      *Identify regional cells given centers and radiuses*

---

**Description**

Identify regional cells given centers and radiuses

**Usage**

```
FindRegionalCells(  
  sce,  
  centerID,  
  enhanced = FALSE,  
  radius = 10,  
  avern = 5,  
  doPlot = FALSE,  
  returnPlot = FALSE  
)
```

**Arguments**

|            |                                                                            |
|------------|----------------------------------------------------------------------------|
| sce        | A single cell experiment object.                                           |
| centerID   | One or a vector of spot IDs as centers of ROIs.                            |
| enhanced   | A logical variable for plotting enhanced plot or not. Default is FALSE.    |
| radius     | A number of fixed ROI radius.                                              |
| avern      | A number of the average sites used to compute unit distance, default is 5. |
| doPlot     | A logical variable to specify whether plot the figure or not.              |
| returnPlot | a logical variable to specify whether output the plot or not.              |

**Value**

A list including center spot ID and regional spot IDs.

**Examples**

```
# FindRegionalCells(sce, centerID = "ACGCCTGACACGCGCT-1")
```



```

doPlot = FALSE,
returnPlot = FALSE)
thisID2 <- S4Vectors::metadata(example_sce)$selectCenters$selectID[2]
thisRadius2 <- S4Vectors::metadata(example_sce)$selectCenters$selectRadius[2]
OutRegRes2 <- RegionalST::FindRegionalCells(example_sce,
centerID = thisID2,
radius = thisRadius2,
enhanced = FALSE,
doPlot = FALSE,
returnPlot = FALSE)

Regional1ID <- OutRegRes1$closeID
Regional2ID <- OutRegRes2$closeID
CTS_DE <- GetCellTypeSpecificDE_withProp(example_sce,
Regional1ID = Regional1ID,
Regional2ID = Regional2ID,
n_markers = 10,
angle = 30,
hjust = 0,
size = 3,
padj_filter = 0.05,
doHeatmap = FALSE)

```

---

GetCrossRegionalDE\_raw

*Identify cross-regional differential analysis*

---

## Description

Identify cross-regional differential analysis

## Usage

```

GetCrossRegionalDE_raw(
  sce,
  twoCenter = c(3, 4),
  enhanced = FALSE,
  label = "celltype",
  n_markers = 10,
  logfc.threshold = 0.25,
  angle = 30,
  hjust = 0,
  size = 3,
  min.pct = 0.1,
  padj_filter = 0.05,
  doHeatmap = TRUE
)

```

## Arguments

|           |                                                         |
|-----------|---------------------------------------------------------|
| sce       | A single cell experiment object.                        |
| twoCenter | A vector of two numbers for the interested ROI numbers. |



|                 |                                                                     |
|-----------------|---------------------------------------------------------------------|
| enhanced        | A logical variable for using enhanced data or not.                  |
| label           | A variable name that contains the cell type information.            |
| n_markers       | A number specifying the top DE gene number.                         |
| logfc.threshold | A number for the cutoff threshold of log fold change.               |
| angle           | A number for angle when plotting.                                   |
| hjust           | A number for horizontal justification when plotting.                |
| size            | A number for text font size.                                        |
| min.pct         | A number of minimum percentage specified in the Seurat DE function. |
| padj_filter     | A number for filtering adjusted p values.                           |
| doHeatmap       | Logical variable for whether drawing the heatmap.                   |

**Value**

A list including the top DE genes (topDE), and all DE genes (allDE).

**Examples**

```
data("example_sce")
example_sce <- mySpatialPreprocess(example_sce, platform="Visium")
# I used a very big padj filter here because this is just a toy data
GetCrossRegionalDE_raw(example_sce, twoCenter = c(1,2),
                       min.pct = 0.01, logfc.threshold = 0.01,
                       padj_filter = 0.5)
```

---

GetCrossRegionalDE\_withProp

*Identify cross-regional differential analysis with proportion*

---

**Description**

Identify cross-regional differential analysis with proportion

**Usage**

```
GetCrossRegionalDE_withProp(
  sce,
  twoCenter = c(3, 4),
  label = "celltype",
  n_markers = 10,
  angle = 30,
  hjust = 0,
  size = 3,
  padj_filter = 0.05,
  doHeatmap = TRUE
)
```

**Arguments**

|             |                                                          |
|-------------|----------------------------------------------------------|
| sce         | A single cell experiment object.                         |
| twoCenter   | A vector of two numbers for the interested ROI numbers.  |
| label       | A variable name that contains the cell type information. |
| n_markers   | A number specifying the top DE gene number.              |
| angle       | A number for angle when plotting.                        |
| hjust       | A number for horizontal justification when plotting.     |
| size        | A number for text font size.                             |
| padj_filter | A number for filtering adjusted p values.                |
| doHeatmap   | Logical variable for whether drawing the heatmap.        |

**Value**

A list including the top DE genes (topDE), and all DE genes (allDE).

**Examples**

```
data("example_sce")
example_sce <- mySpatialPreprocess(example_sce, platform="Visium")
# Since the example data is very small, I set padj filter as NULL. Default is 0.05.
GetCrossRegionalDE_withProp(example_sce, twoCenter = c(1,2), padj_filter = NULL)
```

---

GetOneRadiusEntropy     *Computer the entropy for a fixed radius*

---

**Description**

Computer the entropy for a fixed radius

**Usage**

```
GetOneRadiusEntropy(
  sce,
  selectN,
  enhanced = FALSE,
  weight = NULL,
  label = "celltype",
  radius = 10,
  doPlot = FALSE,
  mytitle = NULL
)
```

**Arguments**

|          |                                                                                    |
|----------|------------------------------------------------------------------------------------|
| sce      | A single cell experiment object.                                                   |
| selectN  | A total number for selected centers. Should be smaller than the total site number. |
| enhanced | A logical variable of whether using enhanced data.                                 |
| weight   | A data frame to specify the weights of all cell types.                             |
| label    | A variable name that contains the cell type information.                           |
| radius   | A number for fixed radius.                                                         |
| doPlot   | Logical variable about whether draw the plot.                                      |
| mytitle  | A character string for the title of the plot.                                      |

**Value**

A list including the selected centers, computed entropies, radius.

**Examples**

```
data("example_sce")
weight <- data.frame(celltype = c("Cancer Epithelial", "CAFs",
                                "T-cells", "Endothelial",
                                "PVL", "Myeloid", "B-cells",
                                "Normal Epithelial", "Plasmablasts"),
                    weight = c(0.25,0.05,
                               0.25,0.05,
                               0.025,0.05,
                               0.25,0.05,0.025))

example_sce <- mySpatialPreprocess(example_sce, platform="Visium")
example_sce$array_col" <- example_sce$col
example_sce$array_row" <- example_sce$row
example_sce$pxl_col_in_fullres" <- example_sce$imagecol
example_sce$pxl_row_in_fullres" <- example_sce$imagerow
GetOneRadiusEntropy(example_sce, selectN = round(length(example_sce$spot)/2),
                    weight = weight, radius = 5, doPlot = FALSE,
                    mytitle = "Radius 5 weighted entropy")
```

---

GetOneRadiusEntropy\_withProp

*Computer the entropy for a fixed radius with cell type proportion*

---

**Description**

Computer the entropy for a fixed radius with cell type proportion

**Usage**

```
GetOneRadiusEntropy_withProp(
  sce,
  selectN,
  weight = NULL,
  label = "celltype",
```

```

radius = 10,
doPlot = FALSE,
mytitle = NULL
)

```

### Arguments

|         |                                                                                    |
|---------|------------------------------------------------------------------------------------|
| sce     | A single cell experiment object.                                                   |
| selectN | A total number for selected centers. Should be smaller than the total site number. |
| weight  | A data frame to specify the weights of all cell types.                             |
| label   | A variable name that contains the cell type information.                           |
| radius  | A number for fixed radius.                                                         |
| doPlot  | Logical variable about whether draw the plot.                                      |
| mytitle | A character string for the title of the plot.                                      |

### Value

A list including the selected centers, computed entropies, radius.

### Examples

```

data("example_sce")
weight <- data.frame(celltype = c("Cancer Epithelial", "CAFs", "T-cells", "Endothelial",
                                "PVL", "Myeloid", "B-cells", "Normal Epithelial", "Plasmablasts"),
                    weight = c(0.25,0.05,
                                0.25,0.05,
                                0.025,0.05,
                                0.25,0.05,0.025))
example_sce <- mySpatialPreprocess(example_sce, platform="Visium")
example_sce$array_col <- example_sce$col
example_sce$array_row <- example_sce$row
example_sce$pxl_col_in_fullres <- example_sce$imagecol
example_sce$pxl_row_in_fullres <- example_sce$imagerow
GetOneRadiusEntropy_withProp(example_sce, selectN = round(length(example_sce$spot)/10),
                             weight = weight,
                             radius = 5,
                             doPlot = TRUE,
                             mytitle = "Radius 5 weighted entropy")

```

---

getProportion

*Define an accessor method for Proportion\_CARD*

---

### Description

Define an accessor method for Proportion\_CARD

### Usage

```
getProportion(card)
```

**Arguments**

card            A CARD object.

**Value**

A matrix containing the spot-level cell type proportion information

**Examples**

```
# getProportion(card)
```

---

ManualSelectCenter    *Manually select top ROIs*

---

**Description**

Manually select top ROIs

**Usage**

```
ManualSelectCenter(sce)
```

**Arguments**

sce            A single cell experiment object.

**Value**

An sce object with selected centers and radiuses.

**Examples**

```
data("example_sce")
example_sce <- mySpatialPreprocess(example_sce, platform="Visium")
# I commented this out because the shiny app will get stuck without input.
# example_sce <- ManualSelectCenter(example_sce)
```

---

mySpatialPreprocess     *Perform Preprocessing for spatial data (tailored from BayesSpace function)*

---

**Description**

Perform Preprocessing for spatial data (tailored from BayesSpace function)

**Usage**

```
mySpatialPreprocess(  
  sce,  
  platform = c("Visium", "ST"),  
  n.PCs = 15,  
  n.HVGs = 2000,  
  skip.PCA = FALSE,  
  assay.type = "logcounts"  
)
```

**Arguments**

|            |                                                                    |
|------------|--------------------------------------------------------------------|
| sce        | A SingleCellExperiment object.                                     |
| platform   | Which platform the data are from, Visium or ST.                    |
| n.PCs      | Number of PCs used in the analysis.                                |
| n.HVGs     | Number of highly variable genes used in the analysis.              |
| skip.PCA   | A boolean variable to choose whether skipping the PCA step or not. |
| assay.type | Which assay to use, default is logcounts.                          |

**Value**

A processed SingleCellExperiment object.

**Examples**

```
data(example_sce)  
example_sce <- mySpatialPreprocess(example_sce, platform="Visium")
```

---

pathways\_hallmark     *Hallmark database*

---

**Description**

Hallmark database downloaded from MSigDB (Feb, 2023)

**Usage**

```
data(pathways_hallmark)
```

**Format**

A list object.

**Value**

A list object.

**Source**

[MSigDB](#)

**References**

Liberzon et al. (2015) Cell Syst. 1(6):417-425 ([PubMed](#))

**Examples**

```
data(pathways_hallmark)
```

---

|               |                      |
|---------------|----------------------|
| pathways_kegg | <i>KEGG database</i> |
|---------------|----------------------|

---

**Description**

KEGG database downloaded from MSigDB (Feb, 2023)

**Usage**

```
data(pathways_kegg)
```

**Format**

A list object.

**Value**

A list object.

**Source**

[MSigDB](#)

**References**

Kanehisa and Goto (2000) Nucleic Acids Research 28(1):27-30 ([PubMed](#))

**Examples**

```
data(pathways_kegg)
```

pathways\_reactome      *REACTOME database*

---

**Description**

REACTOME database downloaded from MSigDB (Feb, 2023)

**Usage**

```
data(pathways_reactome)
```

**Format**

A list object.

**Value**

A list object.

**Source**

[MSigDB](#)

**References**

Jassal et al. (2020) Nucleic Acids Research 28(1):27-30 ([PubMed](#))

**Examples**

```
data(pathways_reactome)
```

---

PlotOneSelectedCenter      *Plot one selected ROI*

---

**Description**

Plot one selected ROI

**Usage**

```
PlotOneSelectedCenter(sce, ploti, enhanced = FALSE)
```

**Arguments**

|          |                                                    |
|----------|----------------------------------------------------|
| sce      | A single cell experiment object.                   |
| ploti    | A number of indicate which ROI to plot.            |
| enhanced | A logical variable for using enhanced data or not. |

**Value**

A figure object for the selected ROI.



**Examples**

```
data("example_sce")
example_sce <- mySpatialPreprocess(example_sce, platform="Visium")
example_sce$array_col <- example_sce$col
example_sce$array_row <- example_sce$row
example_sce$pxl_col_in_fullres <- example_sce$imagecol
example_sce$pxl_row_in_fullres <- example_sce$imagerow
PlotOneSelectedCenter(example_sce, ploti = 1)
```

---

RankCenterByEntropy     *Automatically rank ROI centers based on entropy*

---

**Description**

Automatically rank ROI centers based on entropy

**Usage**

```
RankCenterByEntropy(
  sce,
  weight,
  enhanced = FALSE,
  selectN = round(length(sce$spot)/10),
  label = "celltype",
  topN = 10,
  min_radius = 10,
  avern = 5,
  radius_vec = c(10, 15, 20),
  doPlot = TRUE
)
```

**Arguments**

|            |                                                                                    |
|------------|------------------------------------------------------------------------------------|
| sce        | A single cell experiment object.                                                   |
| weight     | A data frame to specify the weights of all cell types.                             |
| enhanced   | A logical variable of whether using enhanced data.                                 |
| selectN    | A total number for selected centers. Should be smaller than the total site number. |
| label      | A variable name that contains the cell type information.                           |
| topN       | A number to specify the total amount of top ranked ROIs.                           |
| min_radius | The minimum repellent radius.                                                      |
| avern      | A number of the average sites used to compute unit distance, default is 5.         |
| radius_vec | A vector of numbers for candidate radiuses.                                        |
| doPlot     | Logical variable about whether draw the plot.                                      |

**Value**

An sce object with selected ROI information.

**Examples**

```

data("example_sce")
example_sce <- mySpatialPreprocess(example_sce, platform="Visium")
weight <- data.frame(celltype = c("Cancer Epithelial", "CAFs", "T-cells", "Endothelial",
                                "PVL", "Myeloid", "B-cells", "Normal Epithelial", "Plasmablasts"),
                    weight = c(0.25,0.05,
                               0.25,0.05,
                               0.025,0.05,
                               0.25,0.05,0.025))
example_sce <- mySpatialPreprocess(example_sce, platform="Visium")
example_sce$array_col <- example_sce$col
example_sce$array_row <- example_sce$row
example_sce$pxl_col_in_fullres <- example_sce$imagecol
example_sce$pxl_row_in_fullres <- example_sce$imagerow
example_sce <- RankCenterByEntropy(example_sce, weight, label = "celltype",
                                  selectN = round(length(example_sce$spot)/10),
                                  topN = 3, min_radius = 10,
                                  radius_vec = c(10,15),
                                  doPlot = TRUE)

```

---

RankCenterByEntropy\_withProp

*Automatically rank ROI centers based on entropy with proportions*


---

**Description**

Automatically rank ROI centers based on entropy with proportions

**Usage**

```

RankCenterByEntropy_withProp(
  sce,
  weight,
  selectN = round(length(sce$spot)/10),
  topN = 10,
  min_radius = 10,
  avern = 5,
  radius_vec = c(10, 15, 20),
  doPlot = TRUE
)

```

**Arguments**

|            |                                                                                    |
|------------|------------------------------------------------------------------------------------|
| sce        | A single cell experiment object.                                                   |
| weight     | A data frame to specify the weights of all cell types.                             |
| selectN    | A total number for selected centers. Should be smaller than the total site number. |
| topN       | A number to specify the total amount of top ranked ROIs.                           |
| min_radius | The minimum repellent radius.                                                      |
| avern      | A number of the average sites used to compute unit distance, default is 5.         |
| radius_vec | A vector of numbers for candidate radiuses.                                        |
| doPlot     | Logical variable about whether draw the plot.                                      |



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