# ${\bf Package~`Single Cell Complex Heat Map'}$

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Туре	Package
Title	Complex Heatmaps for Single Cell Expression Data with Dual Information Display
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Descr	display gene expression levels (as color intensity) and expression percentages (as circle sizes). Supports gene grouping, cell type annotations, and time point comparisons. Built on top of 'ComplexHeatmap' and integrates with 'Seurat' objects. For more details see Gu (2022) <doi:10.1002 imt2.43=""> and Hao (2024) <doi:10.1038 s41587-023-01767-y="">.</doi:10.1038></doi:10.1002>
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2 create\_cell\_annotations

```
create_cell_annotations
```

Create Cell Type and Time Point Annotations for Heatmap Columns

## **Description**

Parses column names to extract time points and cell types, creates annotations and reorders matrices.

### Usage

```
create_cell_annotations(
  exp_mat,
  percent_mat,
  split_pattern = "_",
  time_position = 1,
  celltype_start = 2,
  time_points_order = NULL,
  cell_types_order = NULL,
  time_color_palette = "Accent",
  celltype_color_palette = "Dark2",
  show_time_annotation = TRUE,
  show_celltype_annotation = TRUE)
```

## **Arguments**

```
Expression matrix with samples as columns
exp_mat
                 Percentage matrix with samples as columns
percent_mat
split_pattern
                 Character string used to split column names (default: "_")
time_position
                  Integer indicating position of time point in split names (default: 1)
celltype_start
                 Integer indicating starting position of cell type in split names (default: 2)
time_points_order
                 Character vector specifying order of time points (default: NULL for automatic)
cell_types_order
                 Character vector specifying order of cell types (default: NULL for automatic)
time_color_palette
                 Character string specifying RColorBrewer palette for time points (default: "Ac-
                 cent")
celltype_color_palette
                 Character string specifying RColorBrewer palette for cell types (default: "Dark2")
show_time_annotation
                 Logical indicating whether to show time point annotation (default: TRUE)
show_celltype_annotation
                 Logical indicating whether to show cell type annotation (default: TRUE)
```

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#### Value

A list containing exp\_mat\_ordered (reordered expression matrix), percent\_mat\_ordered (reordered percentage matrix), col\_annotation (ComplexHeatmap column annotation object), col\_split\_factor (factor for column splitting based on time points), and annotation\_df (data frame with column annotations).

#### See Also

```
create_single_cell_complex_heatmap, prepare_expression_matrices
```

## **Examples**

```
## Not run:
# Prepare expression matrices first
matrices <- prepare_expression_matrices(seurat_obj, features, group_by = "timepoint_cellt</pre>
# Create cell annotations with custom ordering
col_annotations <- create_cell_annotations(</pre>
  exp_mat = matrices$exp_mat,
  percent_mat = matrices$percent_mat,
  split_pattern = "_",
  time_points_order = c("0h", "6h", "12h", "24h"),
  cell_types_order = c("T_cell", "B_cell", "Monocyte"),
 time_color_palette = "Set2",
  celltype_color_palette = "Dark2"
# Access results
ordered_exp_mat <- col_annotations$exp_mat_ordered</pre>
col_annotation <- col_annotations$col_annotation</pre>
col_split_factor <- col_annotations$col_split_factor</pre>
## End(Not run)
```

```
create_gene_annotations
```

Create Gene Group Annotations for Heatmap Rows

## Description

Creates gene grouping annotations and reorders expression matrices based on gene classifications.

### Usage

```
create_gene_annotations(
  exp_mat,
  percent_mat,
  gene_classification,
  color_palette = "Set1",
  sort_within_groups = TRUE
)
```

#### **Arguments**

```
exp_mat Expression matrix with genes as rows

percent_mat Percentage matrix with genes as rows

gene_classification
    Named list where names are group labels and values are character vectors of gene names

color_palette
    Character string specifying RColorBrewer palette name (default: "Set1")

sort_within_groups
    Logical indicating whether to sort genes within each group (default: TRUE)
```

#### Value

A list containing exp\_mat\_ordered (reordered expression matrix), percent\_mat\_ordered (reordered percentage matrix), row\_annotation (ComplexHeatmap row annotation object), row\_split\_factor (factor for row splitting), and annotation\_df (data frame with gene annotations).

#### See Also

```
create_single_cell_complex_heatmap, prepare_expression_matrices
```

### **Examples**

```
## Not run:
# Prepare expression matrices first
matrices <- prepare_expression_matrices(seurat_obj, features, group_by = "cell_type")</pre>
# Define gene groups
gene_groups <- list(</pre>
  "Immune_Response" = c("Gene1", "Gene2"),
  "Cell_Cycle" = c("Gene3", "Gene4"),
  "Metabolism" = c("Gene5", "Gene6")
# Create gene annotations
annotations <- create_gene_annotations(</pre>
  exp_mat = matrices$exp_mat,
  percent_mat = matrices$percent_mat,
  gene_classification = gene_groups,
  color_palette = "Set1"
# Access results
ordered_exp_mat <- annotations$exp_mat_ordered</pre>
ordered_percent_mat <- annotations$percent_mat_ordered</pre>
row_annotation <- annotations$row_annotation</pre>
## End(Not run)
```

```
create_single_cell_complex_heatmap

Create Complex Heatmap for Single Cell Expression Data
```

#### **Description**

Creates a complex heatmap that displays both gene expression levels (as color intensity) and expression percentages (as circle sizes) for single cell RNA-seq data. This function provides extensive customization options while maintaining ease of use.

## Usage

```
create_single_cell_complex_heatmap(
  seurat_object,
  features,
 gene_classification = NULL,
  group_by = "seurat_clusters",
  idents = NULL,
  time_points_order = NULL,
  cell_types_order = NULL,
  color range = c(-1, 0, 2),
  color palette = NULL,
 max_circle_size = 2,
  row_fontsize = 8,
  col fontsize = 9,
  col_name_rotation = 90,
  row_title_fontsize = 10,
  col_title_fontsize = 10,
  show_heatmap_legend = TRUE,
  show_percentage_legend = TRUE,
  legend_side = "right",
  cell_border_color = "grey80",
  split_pattern = "_",
  gene_color_palette = "Set1",
  time_color_palette = "Accent",
  celltype_color_palette = "Dark2",
  show_gene_grouping = NULL,
  show_time_annotation = TRUE,
  show_celltype_annotation = TRUE,
  split_by = "time",
 merge_legends = TRUE,
 percentage_legend_title = "Expression %",
 percentage_legend_labels = c("0%", "25%", "50%", "75%", "100%"),
 percentage breaks = NULL,
  return_data = FALSE,
  save_plot = NULL,
  plot_width = 10,
 plot_height = 8,
 plot_dpi = 300,
  assay = NULL,
  slot = "scale.data",
```

```
cluster cells = TRUE,
      cluster features = TRUE,
      clustering_distance_rows = "euclidean",
      clustering_distance_cols = "euclidean",
      clustering_method_rows = "complete",
      clustering_method_cols = "complete",
      color_palette_main = c("blue", "white", "red"),
      annotation_colors = NULL,
      show_feature_names = TRUE,
      feature_names_gp = NULL,
      legend_title = "Expression",
    )
Arguments
    seurat_object
                    A Seurat object containing single cell data
                    Character vector of gene names to plot
    features
   gene_classification
                    Named list where names are group labels and values are character vectors of
                    gene names (default: NULL for no gene grouping)
                    Character string specifying the metadata column to group by (default: "seu-
   group_by
                    rat clusters")
                    Numeric or character vector specifying which cell groups to include (default:
    idents
                    NULL for all)
   time_points_order
                    Character vector specifying order of time points. Only affects display order, not
                    data filtering (default: NULL for automatic)
    cell_types_order
                    Character vector specifying order of cell types. Only affects display order, not
                    data filtering (default: NULL for automatic)
                    Numeric vector specifying color mapping break points for expression values. Its
    color_range
                    length must match color_palette if color_palette is a vector. (default: c(-1, 0, 2))
    color_palette
                    Character vector specifying colors for expression heatmap. Its length must
                    match color_range. If NULL, a default palette (viridis or color_palette_main) is
                    generated to match color_range length (default: NULL)
   max_circle_size
                    Numeric specifying maximum circle radius in mm. This applies to the highest
                    percentage value in percentage_breaks (default: 2)
    row_fontsize Numeric specifying row name font size (default: 8)
    col_fontsize Numeric specifying column name font size (default: 9)
    col_name_rotation
                    Numeric specifying column name rotation angle (default: 90)
    row_title_fontsize
                    Numeric specifying row title font size (default: 10)
   col_title_fontsize
```

Numeric specifying column title font size (default: 10)

```
show_heatmap_legend
                 Logical indicating whether to show heatmap legend (default: TRUE)
show percentage legend
                 Logical indicating whether to show percentage legend (default: TRUE)
legend_side Character string specifying legend position (default: "right")
cell border color
                 Character string specifying cell border color (default: "grey80")
split_pattern
                 Character string used to split column names for parsing (default: "_")
gene_color_palette
                 Character string specifying RColorBrewer palette for gene groups (default: "Set1")
time_color_palette
                 Character string specifying RColorBrewer palette for time points (default: "Ac-
                 cent")
celltype_color_palette
                 Character string specifying RColorBrewer palette for cell types (default: "Dark2")
show_gene_grouping
                 Logical indicating whether to show gene grouping (default: TRUE if gene_classification
                 provided)
show_time_annotation
                 Logical indicating whether to show time point annotation (default: TRUE)
show_celltype_annotation
                 Logical indicating whether to show cell type annotation (default: TRUE)
                 Character string specifying how to split columns: "time", "celltype", or "none"
split_by
                 (default: "time")
merge_legends
                 Logical indicating whether to merge legends (default: TRUE)
percentage_legend_title
                 Character string for percentage legend title (default: "Expression %")
percentage_legend_labels
                 Character vector for percentage legend labels
percentage_breaks
                 Numeric vector specifying actual percentage values corresponding to labels
return_data Logical; if TRUE, return underlying data instead of drawing only
                 File path to save the drawn heatmap (PNG)
save plot
plot_width
                 Numeric; width in inches for saving
plot_height Numeric; height in inches for saving
plot_dpi
                 Numeric; resolution (DPI) for saved plot
assay
                 Seurat assay name to extract data from
                 Seurat slot name within assay (e.g., "scale.data", "data")
slot
cluster_cells
                 Logical; whether to cluster columns (cells)
cluster_features
                 Logical; whether to cluster rows (features)
clustering_distance_rows
                 Distance metric for row clustering
```

```
clustering_distance_cols
                Distance metric for column clustering
clustering_method_rows
                Clustering method for rows
clustering_method_cols
                Clustering method for columns
color_palette_main
                Fallback color palette when viridis unavailable
annotation_colors
                Named list of custom annotation colors
show_feature_names
                Logical; whether to show feature (row) names
feature_names_gp
                gpar object controlling feature name appearance
legend_title Character; title for main heatmap legend
                Additional arguments passed to ComplexHeatmap::Heatmap()
```

#### Value

A ComplexHeatmap object. If return\_data is TRUE, returns a list containing the heatmap object and underlying data matrices.

```
prepare_expression_matrices
```

Prepare Expression and Percentage Matrices from Seurat DotPlot

# Description

Extracts and reshapes expression data from a Seurat DotPlot object into matrices suitable for complex heatmap visualization.

#### Usage

```
prepare_expression_matrices(
   seurat_object,
   features,
   group_by = "seurat_clusters",
   idents = NULL,
   split_pattern = "_",
   time_position = 1,
   celltype_start = 2
)
```

## **Arguments**

```
A Seurat object

A Seurat object containing single cell data

features

Character vector of gene names to plot

group_by

Character string specifying the metadata column to group by (default: "seurat_clusters")
```

```
idents Numeric or character vector specifying which cell groups to include (default: NULL for all)

split_pattern
Character string used to split column names for parsing (default: "_")

time_position
Integer indicating position of time point in split names (default: 1)

celltype_start
Integer indicating starting position of cell type in split names (default: 2)
```

## Value

A list containing exp\_mat (matrix of scaled expression values), percent\_mat (matrix of expression percentages), and dotplot\_data (original DotPlot data frame).

#### See Also

```
create_single_cell_complex_heatmap
```

#### **Examples**

```
## Not run:
# Basic usage
matrices <- prepare_expression_matrices(</pre>
  seurat_object = my_seurat,
  features = c("Gene1", "Gene2", "Gene3"),
  group_by = "cell_type"
# Advanced usage with specific cell groups
matrices <- prepare_expression_matrices(</pre>
  seurat_object = my_seurat,
  features = gene_list,
  group_by = "timepoint_celltype",
 idents = c("0h_T_cell", "6h_T_cell", "12h_T_cell"),
  split_pattern = "_"
# Access the results
expression_matrix <- matrices$exp_mat</pre>
percentage_matrix <- matrices$percent_mat</pre>
original_data <- matrices$dotplot_data</pre>
## End(Not run)
```