Package 'spatialLIBD'

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Title LIBD Visium spatial transcriptomics human pilot data inspector

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Description Inspect interactively the spatial transcriptomics 10x Genomics Visium data from Maynard, Collado-Torres et al, 2020 analyzed by Lieber Institute for Brain Development researchers and collaborators.

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BugReports https://support.bioconductor.org/t/spatialLIBD

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R topics documented:

check_image_path *Check input image_path*

Description

This function checks that the `image_path` vector has the appropriate structure. For more details please check the vignette documentation.

Usage

```
check_image_path(image_path, spe)
```

Arguments

`image_path`	A path to the directory containing the low resolution histology images that is needed for the interactive visualizations with `plotly`. See https://github.com/LieberInstitute/spatialLIBD/ for an example of how these files should be organized.
`spe`	Defaults to the output of `fetch_data(type = 'spe')`. This is a SpatialExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.

Value

The input object if all checks are passed.

See Also

Other Check input functions: `check_modeling_results()`, `check_sce_layer()`, `check_sce()`

Examples

```
if (enough_ram()) {
    ## Obtain the necessary data
    if (!exists("spe")) spe <- fetch_data("spe")

    ## Get the path to the images
    img_path <- system.file("app", "www", "data", package = "spatialLIBD")

    ## Check the object
    check_image_path(img_path, spe)
}
```

`check_modeling_results` *Check input modeling_results*

Description

This function checks that the `modeling_results` object has the appropriate structure. For more details please check the vignette documentation.

Usage

```
check_modeling_results(modeling_results)
```

Arguments

`modeling_results`	Defaults to the output of `fetch_data(type = 'modeling_results')`. This is a list of tables with the columns f_stat_* or t_stat_* as well as p_value_* and fdr_* plus `ensembl`. The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the `ensembl` column is used for matching in some cases. See `fetch_data()` for more details.

Value

The input object if all checks are passed.

See Also

Other Check input functions: `check_image_path()`, `check_sce_layer()`, `check_sce()`

Examples

```
if (!exists("modeling_results")) {
      modeling_results <- fetch_data(type = "modeling_results")
  }

## Check the object
xx <- check_modeling_results(modeling_results)
```

check_sce *Check input sce*

Description

This function checks that the sce object has the appropriate structure. For more details please check the vignette documentation.

Usage

```
check_sce(
  sce,
 variables = c("GraphBased", "Layer", "Maynard", "Martinowich", paste0("SNN_k50_k",
    4:28), "layer_guess_reordered_short", "cell_count", "sum_umi", "sum_gene",
  "expr_chrM", "expr_chrM_ratio", "SpatialDE_PCA", "SpatialDE_pool_PCA", "HVG_PCA",
  "pseudobulk_PCA", "markers_PCA", "SpatialDE_UMAP", "SpatialDE_pool_UMAP", "HVG_UMAP",
    "pseudobulk_UMAP", "markers_UMAP", "SpatialDE_PCA_spatial",
    "SpatialDE_pool_PCA_spatial", "HVG_PCA_spatial", "pseudobulk_PCA_spatial",
  "markers_PCA_spatial", "SpatialDE_UMAP_spatial", "SpatialDE_pool_UMAP_spatial",
    "HVG_UMAP_spatial", "pseudobulk_UMAP_spatial", "markers_UMAP_spatial")
)
```

Arguments

sce	Defaults to the output of `fetch_data(type = 'sce')`. This is a SingleCellExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.
variables	A `character()` vector of variable names expected to be present in `colData(sce)`.

Value

The input object if all checks are passed.

See Also

Other Check input functions: `check_image_path()`, `check_modeling_results()`, `check_sce_layer()`

Examples

```
if (enough_ram()) {
    ## Obtain the necessary data
    if (!exists("sce")) sce <- fetch_data("sce")

    ## Check the object
    check_sce(sce)
}
```

check_sce_layer *Check input sce_layer*

Description

This function checks that the sce_layer object has the appropriate structure. For more details please check the vignette documentation.

Usage

```
check_sce_layer(sce_layer, variables = "layer_guess_reordered_short")
```

Arguments

sce_layer	Defaults to the output of fetch_data(type = 'sce_layer'). This is a SingleCellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See fetch_data() for more details.
variables	A character() vector of variable names expected to be present in colData(sce_layer).

Value

The input object if all checks are passed.

See Also

Other Check input functions: check_image_path(), check_modeling_results(), check_sce()

Examples

```
## Obtain the necessary data
if (!exists("sce_layer")) sce_layer <- fetch_data("sce_layer")

## Check the object
check_sce_layer(sce_layer)
```

enough_ram *Determine if you have enough RAM memory*

Description

This function determines if you have enough RAM memory on your system.

Usage

```
enough_ram(how_much = 3e+09)
```

Arguments

how_much	The number of bytes you want to compare against.

Details

If benchmarkme::get_ram() fails, this function will return FALSE as a save bet.

Value

A logical(1) indicating whether your system has enough RAM memory.

Examples

```
## Do you have ~ 3 GB in your system?
enough_ram(3e9)

## Do you have ~ 100 GB in your system
enough_ram(100e9)
```

---

fetch_data *Download the Human DLPFC Visium data from LIBD*

Description

This function downloads from ExperimentHub the dorsolateral prefrontal cortex (DLPFC) human Visium data and results analyzed by LIBD. If ExperimentHub is not available, it will download the files from Dropbox using utils::download.file() unless the files are present already at destdir. Note that ExperimentHub will cache the data and automatically detect if you have previously downloaded it, thus making it the preferred way to interact with the data.

Usage

```
fetch_data(
  type = c("sce", "sce_layer", "modeling_results", "sce_example", "spe"),
  destdir = tempdir(),
  eh = ExperimentHub::ExperimentHub(),
  bfc = BiocFileCache::BiocFileCache()
)
```

Arguments

type	A character(1) specifying which file you want to download. It can either be: sce for the SingleCellExperiment object containing the spot-level data that includes the information for visualizing the clusters/genes on top of the Visium histology, sce_layer for the SingleCellExperiment object containing the layer-level data (pseudo-bulked from the spot-level), or modeling_results for the list of tables with the enrichment, pairwise, and anova model results from the layer-level data. It can also be sce_example which is a reduced version

of sce just for example purposes. As of BioC version 3.13 spe downloads a SpatialExperiment-class object.

destdir — The destination directory to where files will be downloaded to in case the ExperimentHub resource is not available. If you already downloaded the files, you can set this to the current path where the files were previously downloaded to avoid re-downloading them.

eh — An ExperimentHub object ExperimentHub-class.

bfc — A BiocFileCache object BiocFileCache-class. Used when eh is not available.

Details

The data was initially prepared by scripts at https://github.com/LieberInstitute/HumanPilot and further refined by https://github.com/LieberInstitute/spatialLIBD/blob/master/inst/scripts/make-data_spatialLIBD.R.

Value

The requested object: sce, sce_layer, ve or modeling_results that you have to assign to an object. If you didn't you can still avoid re-loading the object by using .Last.value.

Examples

```
## Download the SingleCellExperiment object
## at the layer-level
if (!exists("sce_layer")) sce_layer <- fetch_data("sce_layer")

## Explore the data
sce_layer
```

---

gene_set_enrichment *Evaluate the enrichment for a list of gene sets*

Description

Using the layer-level (group-level) data, this function evaluates whether list of gene sets (Ensembl gene IDs) are enrichment among the significant genes (FDR < 0.1 by default) genes for a given model type result.

Usage

```
gene_set_enrichment(
  gene_list,
  fdr_cut = 0.1,
  modeling_results = fetch_data(type = "modeling_results"),
  model_type = names(modeling_results)[1],
  reverse = FALSE
)
```

Arguments

gene_list	A named list object (could be a data.frame) where each element of the list is a character vector of Ensembl gene IDs.
fdr_cut	A numeric(1) specifying the FDR cutoff to use for determining significance among the
modeling_results	Defaults to the output of fetch_data(type = 'modeling_results'). This is a list of tables with the columns f_stat_* or t_stat_* as well as p_value_* and fdr_* plus ensembl. The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the ensembl column is used for matching in some cases. See fetch_data() for more details.
model_type	A named element of the modeling_results list. By default that is either enrichment for the model that tests one human brain layer against the rest (one group vs the rest), pairwise which compares two layers (groups) denoted by layerA-layerB such that layerA is greater than layerB, and anova which determines if any layer (group) is different from the rest adjusting for the mean expression level. The statistics for enrichment and pairwise are t-statistics while the anova model ones are F-statistics.
reverse	A logical(1) indicating whether to multiply by -1 the input statistics and reverse the layerA-layerB column names (using the -) into layerB-layerA.

Details

Check https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/check_clinical_gene_sets.R to see a full script from where this family of functions is derived from.

Value

A table in long format with the enrichment results using stats::fisher.test().

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

See Also

Other Gene set enrichment functions: gene_set_enrichment_plot()

Examples

```
## Read in the SFARI gene sets included in the package
asd_sfari <- utils::read.csv(
    system.file(
        "extdata",
        "SFARI-Gene_genes_01-03-2020release_02-04-2020export.csv",
        package = "spatialLIBD"
    ),
    as.is = TRUE
)
```

```
## Format them appropriately
asd_sfari_geneList <- list(
    Gene_SFARI_all = asd_sfari$ensembl.id,
    Gene_SFARI_high = asd_sfari$ensembl.id[asd_sfari$gene.score < 3],
    Gene_SFARI_syndromic = asd_sfari$ensembl.id[asd_sfari$syndromic == 1]
)

## Obtain the necessary data
if (!exists("modeling_results")) {
      modeling_results <- fetch_data(type = "modeling_results")
  }

## Compute the gene set enrichment results
asd_sfari_enrichment <- gene_set_enrichment(
    gene_list = asd_sfari_geneList,
    modeling_results = modeling_results,
    model_type = "enrichment"
)

## Explore the results
asd_sfari_enrichment
```

---

gene_set_enrichment_plot

Plot the gene set enrichment results

Description

This function takes the output of gene_set_enrichment() and creates a heatmap visualization of the results.

Usage

```
gene_set_enrichment_plot(
  enrichment,
  xlabs = unique(enrichment$ID),
  PThresh = 12,
  ORcut = 3,
  enrichOnly = FALSE,
  layerHeights = c(0, seq_len(length(unique(enrichment$test)))) * 15,
  mypal = c("white", (grDevices::colorRampPalette(RColorBrewer::brewer.pal(9,
    "YlOrRd")))(50)),
  cex = 1.2
)
```

Arguments

enrichment	The output of gene_set_enrichment().
xlabs	A vector of names in the same order and length as unique(enrichment$ID). Gets passed to layer_matrix_plot().
PThresh	A numeric(1) specifying the P-value threshold for the maximum value in the -log10(p) scale.
ORcut	A numeric(1) specifying the P-value threshold for the minimum value in the -log10(p) scale for printing the odds ratio values in the cells of the resulting plot.
enrichOnly	A logical(1) indicating whether to show only odds ratio values greater than 1.
layerHeights	A numeric() vector of length equal to length(unique(enrichment$test)) + 1 that starts at 0 specifying where to plot the y-axis breaks which can be used for re-creating the length of each brain layer. Gets passed to layer_matrix_plot().
mypal	A vector with the color palette to use. Gets passed to layer_matrix_plot().
cex	Passed to layer_matrix_plot().

Details

Check https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/check_clinical_gene_sets.R to see a full script from where this family of functions is derived from.

Value

A plot visualizing the gene set enrichment odds ratio and p-value results.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

See Also

layer_matrix_plot

Other Gene set enrichment functions: gene_set_enrichment()

Examples

```
## Read in the SFARI gene sets included in the package
asd_sfari <- utils::read.csv(
    system.file(
        "extdata",
        "SFARI-Gene_genes_01-03-2020release_02-04-2020export.csv",
        package = "spatialLIBD"
    ),
    as.is = TRUE
)

## Format them appropriately
```

```
asd_sfari_geneList <- list(
    Gene_SFARI_all = asd_sfari$ensembl.id,
    Gene_SFARI_high = asd_sfari$ensembl.id[asd_sfari$gene.score < 3],
    Gene_SFARI_syndromic = asd_sfari$ensembl.id[asd_sfari$syndromic == 1]
)

## Obtain the necessary data
if (!exists("modeling_results")) {
      modeling_results <- fetch_data(type = "modeling_results")
  }

## Compute the gene set enrichment results
asd_sfari_enrichment <- gene_set_enrichment(
    gene_list = asd_sfari_geneList,
    modeling_results = modeling_results,
    model_type = "enrichment"
)

## Visualize the gene set enrichment results
## with a custom color palette
gene_set_enrichment_plot(
    asd_sfari_enrichment,
    xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
    mypal = c(
        "white",
        grDevices::colorRampPalette(
            RColorBrewer::brewer.pal(9, "BuGn")
        )(50)
    )
)

## Specify the layer heights so it resembles more the length of each
## layer in the brain
gene_set_enrichment_plot(
    asd_sfari_enrichment,
    xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
    layerHeights = c(0, 40, 55, 75, 85, 110, 120, 135),
)
```

`geom_spatial` *A ggplot2 layer for visualizing the Visium histology*

Description

This function defines a `ggplot2::layer()` for visualizing the histology image from Visium. It can be combined with other ggplot2 functions for visualizing the clusters as in `vis_clus_p()` or gene-level information as in `vis_gene_p()`.

Usage

```
geom_spatial(
  mapping = NULL,
  data = NULL,
  stat = "identity",
  position = "identity",
  na.rm = FALSE,
  show.legend = NA,
  inherit.aes = FALSE,
  ...
)
```

Arguments

mapping	Passed to ggplot2::layer(mapping) where grob, x and y are required.
data	Passed to ggplot2::layer(data).
stat	Passed to ggplot2::layer(stat).
position	Passed to ggplot2::layer(position).
na.rm	Passed to ggplot2::layer(params = list(na.rm)).
show.legend	Passed to ggplot2::layer(show.legend).
inherit.aes	Passed to ggplot2::layer(inherit.aes).
...	Other arguments passed to ggplot2::layer(params = list(...)).

Value

A ggplot2::layer() for the histology information.

Author(s)

10x Genomics

Examples

```
if (enough_ram()) {
    ## Obtain the necessary data
    if (!exists("spe")) spe <- fetch_data("spe")

    ## Select the first sample and extract the data
    sample_id <- unique(spe$sample_id)[1]
    spe_sub <- spe[, spe$sample_id == sample_id]
    sample_df <- as.data.frame(SpatialExperiment::spatialData(spe_sub))

    ## Make a plot using geom_spatial
    p <- ggplot2::ggplot(
        sample_df,
        ggplot2::aes(
            x = pxl_row_in_fullres * SpatialExperiment::scaleFactors(spe_sub),
            y = pxl_col_in_fullres * SpatialExperiment::scaleFactors(spe_sub),
```

```
        )
    ) +
        geom_spatial(
            data = tibble::tibble(grob = list(SpatialExperiment::imgGrob(spe_sub))),
            ggplot2::aes(grob = grob),
            x = 0.5,
            y = 0.5
        )

    ## Show the plot
    print(p)

    ## Clean up
    rm(spe_sub)
}
```

`get_colors` *Obtain the colors for a set of cluster names*

Description

This function returns a vector of colors based on a vector of cluster names. It can be used to automatically assign colors.

Usage

```
get_colors(colors = NULL, clusters)
```

Arguments

`colors`	A vector of colors. If `NULL` then a set of default colors will be used when `clusters` has less than 12 unique values, otherwise palette36.colors will be used which can generate up to 36 unique colors. If the number of unique clusters is beyond 36 then this function will fail.
`clusters`	A vector of cluster names.

Value

A named vector where the values are the colors to use for displaying them different clusters. For some use cases, you might have to either change the names or use unname().

Examples

```
## Obtain the necessary data
if (!exists("sce_layer")) sce_layer <- fetch_data("sce_layer")

## Example layer colors with the corresponding names
get_colors(libd_layer_colors, sce_layer$layer_guess)
get_colors(libd_layer_colors, sce_layer$layer_guess_reordered_short)
```

```
## Example where colors are assigned automatically
## based on a pre-defined set of colors
get_colors(clusters = sce_layer$kmeans_k7)

## Example where Polychrome::palette36.colors() gets used
get_colors(clusters = letters[seq_len(13)])
```

`layer_boxplot` *Layer-level (group-level) boxplots*

Description

This function uses the output of `sig_genes_extract_all()` as well as the logcounts from the layer-level (group-level) data to visualize the expression of a given gene and display the modeling results for the given gene.

Usage

```
layer_boxplot(
  i = 1,
  sig_genes = sig_genes_extract(),
  short_title = TRUE,
  sce_layer = fetch_data(type = "sce_layer"),
  col_bkg_box = "grey80",
  col_bkg_point = "grey40",
  col_low_box = "violet",
  col_low_point = "darkviolet",
  col_high_box = "skyblue",
  col_high_point = "dodgerblue4",
  cex = 2
)
```

Arguments

`i`	A `integer(1)` indicating which row of `sig_genes` do you want to plot.
`sig_genes`	The output of `sig_genes_extract_all()`.
`short_title`	A `logical(1)` indicating whether to print a short title or not.
`sce_layer`	Defaults to the output of `fetch_data(type = 'sce_layer')`. This is a SingleCellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See `fetch_data()` for more details.
`col_bkg_box`	Box background color for layers not used when visualizing the `pairwise` model results.
`col_bkg_point`	Similar to `col_bkg_box` but for the points.

col_low_box	Box background color for layer(s) with the expected lower expression based on the actual test for row i of sig_genes.
col_low_point	Similar to col_low_box but for the points.
col_high_box	Similar to col_low_box but for the expected layer(s) with higher expression.
col_high_point	Similar to col_high_box but for the points.
cex	Controls the size of the text, points and axis legends.

Value

This function creates a boxplot of the layer-level data (group-level) separated by layer and colored based on the model type from row i of sig_genes.

References

Adapted from https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/layer_specificity.R

See Also

Other Layer modeling functions: sig_genes_extract_all(), sig_genes_extract()

Examples

```
## Obtain the necessary data
if (!exists("modeling_results")) {
      modeling_results <- fetch_data(type = "modeling_results")
  }
if (!exists("sce_layer")) sce_layer <- fetch_data(type = "sce_layer")

## Top 2 genes from the enrichment model
sig_genes <- sig_genes_extract_all(
    n = 2,
    modeling_results = modeling_results,
    sce_layer = sce_layer
)

## Example default boxplot
set.seed(20200206)
layer_boxplot(sig_genes = sig_genes, sce_layer = sce_layer)

## Now show the long title version
set.seed(20200206)
layer_boxplot(
    sig_genes = sig_genes,
    short_title = FALSE,
    sce_layer = sce_layer
)

set.seed(20200206)
layer_boxplot(
    i = which(sig_genes$model_type == "anova")[1],
    sig_genes = sig_genes,
```

```
    sce_layer = sce_layer
)

set.seed(20200206)
layer_boxplot(
    i = which(sig_genes$model_type == "pairwise")[1],
    sig_genes = sig_genes,
    sce_layer = sce_layer
)

## Viridis colors displayed in the shiny app
library("viridisLite")
set.seed(20200206)
layer_boxplot(
    sig_genes = sig_genes,
    sce_layer = sce_layer,
    col_low_box = viridis(4)[2],
    col_low_point = viridis(4)[1],
    col_high_box = viridis(4)[3],
    col_high_point = viridis(4)[4]
)

## Paper colors displayed in the shiny app
set.seed(20200206)
layer_boxplot(
    sig_genes = sig_genes,
    sce_layer = sce_layer,
    col_low_box = "palegreen3",
    col_low_point = "springgreen2",
    col_high_box = "darkorange2",
    col_high_point = "orange1"
)

## Blue/red colors displayed in the shiny app
set.seed(20200206)
layer_boxplot(
    i = which(sig_genes$model_type == "pairwise")[1],
    sig_genes = sig_genes,
    sce_layer = sce_layer,
    col_bkg_box = "grey90",
    col_bkg_point = "grey60",
    col_low_box = "skyblue2",
    col_low_point = "royalblue3",
    col_high_box = "tomato2",
    col_high_point = "firebrick4",
    cex = 3
)
```

`layer_matrix_plot` *Visualize a matrix of values across human brain layers*

Description

This function visualizes a numerical matrix where the Y-axis represents the human brain layers and can be adjusted to represent the length of each brain layer. Cells can optionally have text values. This function is used by `gene_set_enrichment_plot()` and `layer_stat_cor_plot()`.

Usage

```
layer_matrix_plot(
  matrix_values,
  matrix_labels = NULL,
  xlabs = NULL,
  layerHeights = NULL,
  mypal = c("white", (grDevices::colorRampPalette(RColorBrewer::brewer.pal(9,
    "YlOrRd")))(50)),
  breaks = NULL,
  axis.args = NULL,
  srt = 45,
  mar = c(8, 4, 4, 2) + 0.1,
  cex = 1.2
)
```

Arguments

`matrix_values`	A `matrix()` with one column per set of interest and one row per layer (group) with numeric values.
`matrix_labels`	Optionally a character `matrix()` with the same dimensions and `dimnames()` as `matrix_values` with text labels for the cells.
`xlabs`	A vector of names in the same order and length as `colnames(matrix_values)`.
`layerHeights`	A `numeric()` vector of length equal to `nrow(matrix_values) + 1` that starts at 0 specifying where to plot the y-axis breaks which can be used for re-creating the length of each brain layer.
`mypal`	A vector with the color palette to use.
`breaks`	Passed to `fields::image.plot()`. Used by `layer_stat_cor_plot()`.
`axis.args`	Passed to `fields::image.plot()`. Used by `layer_stat_cor_plot()`.
`srt`	The angle for the x-axis labels. Used by `layer_stat_cor_plot()`.
`mar`	Passed to `graphics::par()`.
`cex`	Used for the x-axis labels and the text inside the cells.

Value

A base R plot visualizing the input `matrix_values` with optional text labels for `matrix_labels`.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

Examples

```
## Create some random data
set.seed(20200224)
mat <- matrix(runif(7 * 8, min = -1), nrow = 7)
rownames(mat) <- c("WM", paste0("L", rev(seq_len(6))))
colnames(mat) <- paste0("Var", seq_len(8))

## Create some text labels
mat_text <- matrix("", nrow = 7, ncol = 8, dimnames = dimnames(mat))
diag(mat_text) <- as.character(round(diag(mat), 2))

## Make the plot
layer_matrix_plot(mat, mat_text)

## Try to re-create the anatomical proportions of the human brain layers
layer_matrix_plot(
    mat,
    mat_text,
    layerHeights = c(0, 40, 55, 75, 85, 110, 120, 135),
    cex = 2
)
```

---

layer_stat_cor *Layer modeling correlation of statistics*

Description

Layer modeling correlation of statistics

Usage

```
layer_stat_cor(
  stats,
  modeling_results = fetch_data(type = "modeling_results"),
  model_type = names(modeling_results)[1],
  reverse = FALSE
)
```

Arguments

stats	A data.frame where the row names are Ensembl gene IDs, the column names are labels for clusters of cells or cell types, and where each cell contains the given statistic for that gene and cell type. These statistics should be computed similarly to the modeling results from the data we provide. For example, like the `enrichment` t-statistics that are derived from comparing one layer against the rest. The `stats` will be matched and then correlated with our statistics.

`modeling_results`
: Defaults to the output of `fetch_data(type = 'modeling_results')`. This is a list of tables with the columns f_stat_* or t_stat_* as well as p_value_* and fdr_* plus `ensembl`. The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the `ensembl` column is used for matching in some cases. See `fetch_data()` for more details.

`model_type`
: A named element of the `modeling_results` list. By default that is either `enrichment` for the model that tests one human brain layer against the rest (one group vs the rest), `pairwise` which compares two layers (groups) denoted by `layerA-layerB` such that `layerA` is greater than `layerB`, and `anova` which determines if any layer (group) is different from the rest adjusting for the mean expression level. The statistics for `enrichment` and `pairwise` are t-statistics while the `anova` model ones are F-statistics.

`reverse`
: A `logical(1)` indicating whether to multiply by `-1` the input statistics and reverse the `layerA-layerB` column names (using the `-`) into `layerB-layerA`.

Details

Check https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/dlpfc_snRNAseq_annotation.R for a full analysis from which this family of functions is derived from.

Value

A correlation matrix between `stats` and our statistics using only the Ensembl gene IDs present in both tables. The columns are sorted using a hierarchical cluster.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

See Also

Other Layer correlation functions: `layer_stat_cor_plot()`

Examples

```
## Obtain the necessary data
if (!exists("modeling_results")) {
      modeling_results <- fetch_data(type = "modeling_results")
  }

## Compute the correlations
cor_stats_layer <- layer_stat_cor(
    tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer,
    modeling_results,
    "enrichment"
)

## Explore the correlation matrix
head(cor_stats_layer[, seq_len(3)])
```

`layer_stat_cor_plot` *Visualize the layer modeling correlation of statistics*

Description

This function makes a heatmap from the `layer_stat_cor()` correlation matrix between a given set of cell cluster/type statistics derived from scRNA-seq or snRNA-seq data (among other types) and the layer statistics from the Human DLPFC Visium data (when using the default arguments).

Usage

```
layer_stat_cor_plot(
  cor_stats_layer,
  max = 0.81,
  min = -max,
  layerHeights = NULL,
  cex = 1.2
)
```

Arguments

`cor_stats_layer`	The output of `layer_stat_cor()`.
`max`	A `numeric(1)` specifying the highest correlation value for the color scale (should be between 0 and 1).
`min`	A `numeric(1)` specifying the lowest correlation value for the color scale (should be between 0 and -1).
`layerHeights`	A `numeric()` vector of length equal to `ncol(cor_stats_layer) + 1` that starts at 0 specifying where to plot the y-axis breaks which can be used for re-creating the length of each brain layer. Gets passed to `layer_matrix_plot()`.
`cex`	Passed to `layer_matrix_plot()`.

Details

Check https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/dlpfc_snRNAseq_annotation.R for a full analysis from which this family of functions is derived from.

Value

A heatmap for the correlation matrix between statistics.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

See Also

layer_matrix_plot

Other Layer correlation functions: `layer_stat_cor()`

Examples

```
## Obtain the necessary data
if (!exists("modeling_results")) {
      modeling_results <- fetch_data(type = "modeling_results")
  }

## Compute the correlations
cor_stats_layer <- layer_stat_cor(
    tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer,
    modeling_results,
    "enrichment"
)

## Visualize the correlation matrix
layer_stat_cor_plot(cor_stats_layer)

## Restrict the range of colors
layer_stat_cor_plot(cor_stats_layer, max = 0.3)
```

---

libd_layer_colors *Vector of LIBD layer colors*

Description

A named vector of colors to use for the LIBD layers designed by Lukas M. Weber with feedback from the spatialLIBD collaborators.

Usage

```
libd_layer_colors
```

Format

A vector of length 9 with colors for Layers 1 through 9, WM, NA and a special WM2 that is present in some of the unsupervised clustering results.

run_app *Run the spatialLIBD Shiny Application*

Description

This function runs the shiny application that allows users to interact with the Visium spatial transcriptomics data from LIBD (by default) or any other data that you have shaped according to our object structure.

Usage

```
run_app(
  spe = fetch_data(type = "spe"),
  sce_layer = fetch_data(type = "sce_layer"),
  modeling_results = fetch_data(type = "modeling_results"),
  sig_genes = sig_genes_extract_all(n = nrow(sce_layer), modeling_results =
    modeling_results, sce_layer = sce_layer),
  image_path = system.file("app", "www", "data", package = "spatialLIBD"),
  spe_discrete_vars = c("GraphBased", "Layer", "Maynard", "Martinowich",
   paste0("SNN_k50_k", 4:28), "SpatialDE_PCA", "SpatialDE_pool_PCA", "HVG_PCA",
  "pseudobulk_PCA", "markers_PCA", "SpatialDE_UMAP", "SpatialDE_pool_UMAP", "HVG_UMAP",
    "pseudobulk_UMAP", "markers_UMAP", "SpatialDE_PCA_spatial",
    "SpatialDE_pool_PCA_spatial", "HVG_PCA_spatial", "pseudobulk_PCA_spatial",
  "markers_PCA_spatial", "SpatialDE_UMAP_spatial", "SpatialDE_pool_UMAP_spatial",
    "HVG_UMAP_spatial", "pseudobulk_UMAP_spatial", "markers_UMAP_spatial"),
  spe_continuous_vars = c("cell_count", "sum_umi", "sum_gene", "expr_chrM",
    "expr_chrM_ratio"),
  spatial_libd_var = "layer_guess_reordered_short",
  ...
)
```

Arguments

spe	Defaults to the output of `fetch_data(type = 'spe')`. This is a SpatialExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.
sce_layer	Defaults to the output of `fetch_data(type = 'sce_layer')`. This is a SingleCellExperiment object with the spot-level Visium data compressed via pseudobulking to the layer-level (group-level) resolution. See `fetch_data()` for more details.
modeling_results	Defaults to the output of `fetch_data(type = 'modeling_results')`. This is a list of tables with the columns f_stat_* or t_stat_* as well as p_value_* and fdr_* plus `ensembl`. The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the `ensembl` column is used for matching in some cases. See `fetch_data()` for more details.

sig_genes — The output of sig_genes_extract_all() which is a table in long format with the modeling results.

image_path — A path to the directory containing the low resolution histology images that is needed for the interactive visualizations with plotly. See https://github.com/LieberInstitute/spatialLIBD/ for an example of how these files should be organized.

spe_discrete_vars — A character() vector of discrete variables that will be available to visualize in the app. Basically, the set of variables with spot-level groups. They will have to be present in colData(spe).

spe_continuous_vars — A character() vector of continuous variables that will be available to visualize in the app using the same scale as genes. They will have to be present in colData(sce).

spatial_libd_var — A character(1) with the name of the main cluster variable to use. It will have to be present in both colData(spe) and colData(sce_layer).

... — Other arguments passed to the list of golem options for running the application.

Value

A shiny.appobj that contains the input data.

Examples

```
## Not run:
## The default arguments will download the data from the web
## using fetch_data(). If this is the first time you have run this,
## the files will need to be cached by ExperimentHub. Otherwise it
## will re-use the files you have previously downloaded.
if (enough_ram(4e9)) {
    run_app()
}

## End(Not run)
```

---

sce_to_spe *Convert a SCE object to a SPE one*

Description

This function converts a spot-level SingleCellExperiment-class (SCE) object as generated by fetch_data() to a SpatialExperiment-class (SPE) object.

Usage

```
sce_to_spe(sce = fetch_data("sce"), imageData = NULL)
```

Arguments

sce	Defaults to the output of `fetch_data(type = 'sce')`. This is a SingleCellExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.
imageData	A `DataFrame()` with image data. Will be used with SpatialExperiment::imgData. If `NULL`, then this will be constructed for you assuming that you are working with the original data from `spatialLIBD::fetch_data("sce")`.

Details

Note that the resulting object is a bit more complex than a regular SPE because it contains the data from the spatialLIBD project which you might otherwise have to generate for your own data. See TODO for more information.

Value

A a SpatialExperiment-class object.

Author(s)

Brenda Pardo, Leonardo Collado-Torres

Examples

```
if (enough_ram()) {
    ## Download the sce data
    sce <- fetch_data("sce")
    ## Transform it to a SpatialExperiment object
    spe <- sce_to_spe(sce)
}
```

---

sig_genes_extract *Extract significant genes*

Description

From the layer-level modeling results, this function extracts the top n significant genes. This is the workhorse function used by `sig_genes_extract_all()` through which we obtain the information that can then be used by functions such as `layer_boxplot()` for constructing informative titles.

Usage

```
sig_genes_extract(
  n = 10,
  modeling_results = fetch_data(type = "modeling_results"),
  model_type = names(modeling_results)[1],
  reverse = FALSE,
  sce_layer = fetch_data(type = "sce_layer")
)
```

Arguments

n	The number of the top ranked genes to extract.
modeling_results	Defaults to the output of fetch_data(type = 'modeling_results'). This is a list of tables with the columns f_stat_* or t_stat_* as well as p_value_* and fdr_* plus ensembl. The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the ensembl column is used for matching in some cases. See fetch_data() for more details.
model_type	A named element of the modeling_results list. By default that is either enrichment for the model that tests one human brain layer against the rest (one group vs the rest), pairwise which compares two layers (groups) denoted by layerA-layerB such that layerA is greater than layerB, and anova which determines if any layer (group) is different from the rest adjusting for the mean expression level. The statistics for enrichment and pairwise are t-statistics while the anova model ones are F-statistics.
reverse	A logical(1) indicating whether to multiply by -1 the input statistics and reverse the layerA-layerB column names (using the -) into layerB-layerA.
sce_layer	Defaults to the output of fetch_data(type = 'sce_layer'). This is a SingleCellExperiment object with the spot-level Visium data compressed via pseudobulking to the layer-level (group-level) resolution. See fetch_data() for more details.

Value

A data.frame() with the top n significant genes (as ordered by their statistics in decreasing order) in long format. The specific columns are described further in the vignette.

References

Adapted from https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/layer_specificity_functio

See Also

Other Layer modeling functions: layer_boxplot(), sig_genes_extract_all()

Examples

```
## Obtain the necessary data
if (!exists("modeling_results")) {
      modeling_results <- fetch_data(type = "modeling_results")
  }
if (!exists("sce_layer")) sce_layer <- fetch_data(type = "sce_layer")

## anova top 10 genes
sig_genes_extract(
    modeling_results = modeling_results,
    sce_layer = sce_layer
)
```

```
## Extract all genes
sig_genes_extract(
    modeling_results = modeling_results,
    sce_layer = sce_layer,
    n = nrow(sce_layer)
)
```

`sig_genes_extract_all` *Extract significant genes for all modeling results*

Description

This function combines the output of `sig_genes_extract()` from all the layer-level (group-level) modeling results and builds the data required for functions such as `layer_boxplot()`.

Usage

```
sig_genes_extract_all(
  n = 10,
  modeling_results = fetch_data(type = "modeling_results"),
  sce_layer = fetch_data(type = "sce_layer")
)
```

Arguments

`n`	The number of the top ranked genes to extract.
`modeling_results`	Defaults to the output of `fetch_data(type = 'modeling_results')`. This is a list of tables with the columns f_stat_* or t_stat_* as well as p_value_* and fdr_* plus `ensembl`. The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the `ensembl` column is used for matching in some cases. See `fetch_data()` for more details.
`sce_layer`	Defaults to the output of `fetch_data(type = 'sce_layer')`. This is a SingleCellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See `fetch_data()` for more details.

Value

A DataFrame-class with the extracted statistics in long format. The specific columns are described further in the vignette.

See Also

Other Layer modeling functions: `layer_boxplot()`, `sig_genes_extract()`

Examples

```
## Obtain the necessary data
if (!exists("modeling_results")) {
      modeling_results <- fetch_data(type = "modeling_results")
  }
if (!exists("sce_layer")) sce_layer <- fetch_data(type = "sce_layer")

## top 10 genes for all models
sig_genes_extract_all(
    modeling_results = modeling_results,
    sce_layer = sce_layer
)
```

`sort_clusters` *Sort clusters by frequency*

Description

This function takes a vector with cluster labels and sorts it by frequency such that the most frequent cluster is the first one and so on.

Usage

```
sort_clusters(clusters, map_subset = NULL)
```

Arguments

`clusters`	A vector with cluster labels.
`map_subset`	A logical vector of length equal to `clusters` specifying which elements of `clusters` to use to determine the ranking of the clusters.

Value

A factor of length equal to `clusters` where the levels are the new ordered clusters and the names of the factor are the original values from `clusters`.

Examples

```
## Build an initial set of cluster labels
clus <- letters[unlist(lapply(4:1, function(x) rep(x, x)))]

## In this case, it's a character vector
class(clus)

## Sort them and obtain a factor
sort_clusters(clus)
```

tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer *Cell cluster t-statistics from Tran et al*

Description

Using the DLPFC snRNA-seq data from Matthew N Tran et al we computed enrichment t-statistics for the cell clusters. This is a subset of them used in examples such as in `layer_stat_cor_plot()`.

Usage

```
tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer
```

Format

A matrix with 692 rows and 31 variables where each column is a given cell cluster from Tran et al and each row is one gene. The row names are Ensembl gene IDs which are used by `layer_stat_cor()` to match to our modeling results.

Source

https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/dlpfc_snRNAseq_annotation.R and https://github.com/LieberInstitute/spatialLIBD/blob/master/dev/02_dev.R#L107-L194.

vis_clus *Sample spatial cluster visualization*

Description

This function visualizes the clusters for one given sample at the spot-level using (by default) the histology information on the background. To visualize gene-level (or any continuous variable) use `vis_gene()`.

Usage

```
vis_clus(
  spe,
  sampleid,
  clustervar,
 colors = c("#b2df8a", "#e41a1c", "#377eb8", "#4daf4a", "#ff7f00", "gold", "#a65628",
    "#999999", "black", "grey", "white", "purple"),
  spatial = TRUE,
  ...
)
```

Arguments

spe	Defaults to the output of `fetch_data(type = 'spe')`. This is a SpatialExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.
sampleid	A `character(1)` specifying which sample to plot from `colData(spe)$sample_name`.
clustervar	A `character(1)` with the name of the `colData(spe)` column that has the cluster values.
colors	A vector of colors to use for visualizing the clusters from `clustervar`. If the vector has names, then those should match the values of `clustervar`.
spatial	A `logical(1)` indicating whether to include the histology layer from `geom_spatial()`. If you plan to use ggplotly() then it's best to set this to `FALSE`.
...	Passed to paste0() for making the title of the plot following the `sampleid`.

Details

This function subsets spe to the given sample and prepares the data and title for `vis_clus_p()`.

Value

A ggplot2 object.

See Also

Other Spatial cluster visualization functions: `vis_clus_p()`, `vis_grid_clus()`

Examples

```
if (enough_ram()) {
    ## Obtain the necessary data
    if (!exists("spe")) spe <- fetch_data("spe")

    ## Check the colors defined by Lukas M Weber
    libd_layer_colors

    ## Use the manual color palette by Lukas M Weber
    vis_clus(
        spe = spe,
        clustervar = "layer_guess_reordered",
        sampleid = "151673",
        colors = libd_layer_colors,
        ... = " LIBD Layers"
    )

    ## Without histology
    vis_clus(
        spe = spe,
        clustervar = "layer_guess_reordered",
        sampleid = "151673",
        colors = libd_layer_colors,
```

```
        ... = " LIBD Layers",
        spatial = FALSE
    )
}
```

---

vis_clus_p *Sample spatial cluster visualization workhorse function*

Description

This function visualizes the clusters for one given sample at the spot-level using (by default) the histology information on the background. This is the function that does all the plotting behind `vis_clus()`. To visualize gene-level (or any continuous variable) use `vis_gene_p()`.

Usage

```
vis_clus_p(spe, d, clustervar, sampleid, colors, spatial, title)
```

Arguments

spe	Defaults to the output of `fetch_data(type = 'spe')`. This is a SpatialExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.
d	A data.frame with the sample-level information. This is typically obtained using `spatialData(spe,cd_bind = TRUE,as_df = TRUE)`.
clustervar	A `character(1)` with the name of the `colData(spe)` column that has the cluster values.
sampleid	A `character(1)` specifying which sample to plot from `colData(spe)$sample_name`.
colors	A vector of colors to use for visualizing the clusters from `clustervar`. If the vector has names, then those should match the values of `clustervar`.
spatial	A `logical(1)` indicating whether to include the histology layer from `geom_spatial()`. If you plan to use ggplotly() then it's best to set this to `FALSE`.
title	The title for the plot.

Value

A ggplot2 object.

See Also

Other Spatial cluster visualization functions: `vis_clus()`, `vis_grid_clus()`

Examples

```
if (enough_ram()) {
    ## Obtain the necessary data
    if (!exists("spe")) spe <- fetch_data("spe")
    spe_sub <- spe[, spe$sample_id == "151673"]

    ## Use the manual color palette by Lukas M Weber
    ## Don't plot the histology information
    vis_clus_p(
        spe = spe_sub,
        d = SpatialExperiment::spatialData(spe_sub, cd_bind = TRUE, as_df = TRUE),
        clustervar = "layer_guess_reordered",
        sampleid = "151673",
        colors = libd_layer_colors,
        title = "151673 LIBD Layers",
        spatial = FALSE
    )

    ## Clean up
    rm(spe_sub)
}
```

---

vis_gene *Sample spatial gene visualization*

Description

This function visualizes the gene expression stored in `assays(spe)` or any continuous variable stored in `colData(spe)` for one given sample at the spot-level using (by default) the histology information on the background. To visualize clusters (or any discrete variable) use `vis_clus()`.

Usage

```
vis_gene(
  spe,
  sampleid,
  geneid = "SCGB2A2; ENSG00000110484",
  spatial = TRUE,
  assayname = "logcounts",
  minCount = 0,
  viridis = TRUE,
  ...
)
```

Arguments

spe	Defaults to the output of `fetch_data(type = 'spe')`. This is a SpatialExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.

sampleid	A character(1) specifying which sample to plot from colData(spe)$sample_name.
geneid	A character(1) specifying the gene ID stored in rowData(spe)$gene_search or a continuous variable stored in colData(spe) to visualize.
spatial	A logical(1) indicating whether to include the histology layer from geom_spatial(). If you plan to use ggplotly() then it's best to set this to FALSE.
assayname	The name of the assays(spe) to use for extracting the gene expression data. Defaults to logcounts.
minCount	A numeric(1) specifying the minimum gene expression (or value in the continuous variable) to visualize. Values at or below this threshold will be set to NA. Defaults to 0.
viridis	A logical(1) whether to use the color-blind friendly palette from viridis or the color palette used in the paper that was chosen for contrast when visualizing the data on top of the histology image. One issue is being able to differentiate low values from NA ones due to the purple-ish histology information that is dependent on cell density.
...	Passed to paste0() for making the title of the plot following the sampleid.

Details

This function subsets spe to the given sample and prepares the data and title for vis_gene_p(). It also adds a caption to the plot.

Value

A ggplot2 object.

See Also

Other Spatial gene visualization functions: vis_gene_p(), vis_grid_gene()

Examples

```
if (enough_ram()) {
    ## Obtain the necessary data
    if (!exists("spe")) spe <- fetch_data("spe")

    ## Valid `geneid` values are those in
    head(rowData(spe)$gene_search)
    ## or continuous variables stored in colData(spe)

    ## Visualize a default gene on the non-viridis scale
    vis_gene(
        spe = spe,
        sampleid = "151507",
        viridis = FALSE
    )

    ## Visualize a continuous variable, in this case, the ratio of chrM
    ## gene expression compared to the total expression at the spot-level
```

```
    vis_gene(
        spe = spe,
        sampleid = "151507",
        geneid = "expr_chrM_ratio"
    )
}
```

`vis_gene_p` *Sample spatial gene visualization workhorse function*

Description

This function visualizes the gene expression stored in `assays(spe)` or any continuous variable stored in `colData(spe)` for one given sample at the spot-level using (by default) the histology information on the background. This is the function that does all the plotting behind `vis_gene()`. To visualize clusters (or any discrete variable) use `vis_clus_p()`.

Usage

```
vis_gene_p(spe, d, sampleid, spatial, title, viridis = TRUE)
```

Arguments

`spe`	Defaults to the output of `fetch_data(type = 'spe')`. This is a SpatialExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.
`d`	A data.frame with the sample-level information. This is typically obtained using `spatialData(spe,cd_bind = TRUE,as_df = TRUE)`. The data.frame has to contain a column with the continuous variable data to plot stored under `d$COUNT`.
`sampleid`	A `character(1)` specifying which sample to plot from `colData(spe)$sample_name`.
`spatial`	A `logical(1)` indicating whether to include the histology layer from `geom_spatial()`. If you plan to use ggplotly() then it's best to set this to `FALSE`.
`title`	The title for the plot.
`viridis`	A `logical(1)` whether to use the color-blind friendly palette from viridis or the color palette used in the paper that was chosen for contrast when visualizing the data on top of the histology image. One issue is being able to differentiate low values from NA ones due to the purple-ish histology information that is dependent on cell density.

Value

A ggplot2 object.

See Also

Other Spatial gene visualization functions: `vis_gene()`, `vis_grid_gene()`

Examples

```
if (enough_ram()) {
    ## Obtain the necessary data
    if (!exists("spe")) spe <- fetch_data("spe")

    ## Prepare the data for the plotting function
    spe_sub <- spe[, spe$sample_id == "151673"]
    df <- SpatialExperiment::spatialData(spe_sub, cd_bind = TRUE, as_df = TRUE)
    df$COUNT <- df$expr_chrM_ratio

    ## Use the manual color palette by Lukas M Weber
    ## Don't plot the histology information
    vis_gene_p(
        spe = spe_sub,
        d = df,
        sampleid = "151673",
        title = "151673 chrM expr ratio",
        spatial = FALSE
    )

    ## Clean up
    rm(spe_sub)
}
```

`vis_grid_clus` *Sample spatial cluster visualization grid*

Description

This function visualizes the clusters for a set of samples at the spot-level using (by default) the histology information on the background. To visualize gene-level (or any continuous variable) use `vis_grid_gene()`.

Usage

```
vis_grid_clus(
  spe,
  clustervar,
  pdf_file,
  sort_clust = TRUE,
  colors = NULL,
  return_plots = FALSE,
  spatial = TRUE,
  height = 24,
  width = 36,
  ...
)
```

Arguments

spe	Defaults to the output of `fetch_data(type = 'spe')`. This is a SpatialExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.
clustervar	A `character(1)` with the name of the `colData(spe)` column that has the cluster values.
pdf_file	A `character(1)` specifying the path for the resulting PDF.
sort_clust	A `logical(1)` indicating whether you want to sort the clusters by frequency using `sort_clusters()`.
colors	A vector of colors to use for visualizing the clusters from `clustervar`. If the vector has names, then those should match the values of `clustervar`.
return_plots	A `logical(1)` indicating whether to print the plots to a PDF or to return the list of plots that you can then print using plot_grid.
spatial	A `logical(1)` indicating whether to include the histology layer from `geom_spatial()`. If you plan to use ggplotly() then it's best to set this to `FALSE`.
height	A `numeric(1)` passed to pdf.
width	A `numeric(1)` passed to pdf.
...	Passed to paste0() for making the title of the plot following the `sampleid`.

Details

This function prepares the data and then loops through `vis_clus()` for computing the list of ggplot2 objects.

Value

A list of ggplot2 objects.

See Also

Other Spatial cluster visualization functions: `vis_clus_p()`, `vis_clus()`

Examples

```
if (enough_ram()) {
    ## Obtain the necessary data
    if (!exists("spe")) spe <- fetch_data("spe")

    ## Subset to two samples of interest
    spe_sub <- spe[, spe$sample_id %in% c("151673", "151674")]

    ## Obtain the plot list
    p_list <-
        vis_grid_clus(
            spe_sub,
            "layer_guess_reordered",
            spatial = FALSE,
```

```
            return_plots = TRUE,
            sort_clust = FALSE,
            colors = libd_layer_colors
        )

    ## Clean up
    rm(spe_sub)

    ## Visualize the spatial adjacent replicates for position = 0 micro meters
    ## for subject 3
    cowplot::plot_grid(plotlist = p_list, ncol = 2)
}
```

`vis_grid_gene` *Sample spatial gene visualization grid*

Description

This function visualizes the gene expression stored in `assays(spe)` or any continuous variable stored in `colData(spe)` for a set of samples at the spot-level using (by default) the histology information on the background. To visualize clusters (or any discrete variable) use `vis_grid_clus()`.

Usage

```
vis_grid_gene(
  spe,
  geneid = "SCGB2A2; ENSG00000110484",
  pdf_file,
  assayname = "logcounts",
  minCount = 0,
  return_plots = FALSE,
  spatial = TRUE,
  viridis = TRUE,
  height = 24,
  width = 36,
  ...
)
```

Arguments

`spe`	Defaults to the output of `fetch_data(type = 'spe')`. This is a SpatialExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.
`geneid`	A `character(1)` specifying the gene ID stored in `rowData(spe)$gene_search` or a continuous variable stored in `colData(spe)` to visualize.
`pdf_file`	A `character(1)` specifying the path for the resulting PDF.
`assayname`	The name of the `assays(spe)` to use for extracting the gene expression data. Defaults to `logcounts`.

minCount	A numeric(1) specifying the minimum gene expression (or value in the continuous variable) to visualize. Values at or below this threshold will be set to NA. Defaults to 0.
return_plots	A logical(1) indicating whether to print the plots to a PDF or to return the list of plots that you can then print using plot_grid.
spatial	A logical(1) indicating whether to include the histology layer from geom_spatial(). If you plan to use ggplotly() then it's best to set this to FALSE.
viridis	A logical(1) whether to use the color-blind friendly palette from viridis or the color palette used in the paper that was chosen for contrast when visualizing the data on top of the histology image. One issue is being able to differentiate low values from NA ones due to the purple-ish histology information that is dependent on cell density.
height	A numeric(1) passed to pdf.
width	A numeric(1) passed to pdf.
...	Passed to paste0() for making the title of the plot following the sampleid.

Details

This function prepares the data and then loops through vis_gene() for computing the list of ggplot2 objects.

Value

A list of ggplot2 objects.

See Also

Other Spatial gene visualization functions: vis_gene_p(), vis_gene()

Examples

```
if (enough_ram()) {
    ## Obtain the necessary data
    if (!exists("spe")) spe <- fetch_data("spe")

    ## Subset to two samples of interest
    spe_sub <- spe[, spe$sample_id %in% c("151673", "151674")]

    ## Obtain the plot list
    p_list <-
        vis_grid_gene(
            spe_sub,
            spatial = FALSE,
            return_plots = TRUE
        )

    ## Clean up
    rm(spe_sub)
```

```
    ## Visualize the spatial adjacent replicates for position = 0 micro meters
    ## for subject 3
    cowplot::plot_grid(plotlist = p_list, ncol = 2)
}
```

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