# Package 'ISAnalytics'

August 20, 2025

**Title** Analyze gene therapy vector insertion sites data identified from genomics next generation sequencing reads for clonal tracking studies

**Version** 1.18.0

**Date** 2020-07-03

**Description** In gene therapy, stem cells are modified using viral vectors to deliver the therapeutic transgene and replace functional properties since the genetic modification is stable and inherited in all cell progeny. The retrieval and mapping of the sequences flanking the virus-host DNA junctions allows the identification of insertion sites (IS), essential for monitoring the evolution of genetically modified cells in vivo. A comprehensive toolkit for the analysis of IS is required to foster clonal trackign studies and supporting the assessment of safety and long term efficacy in vivo. This package is aimed at (1) supporting automation of IS workflow, (2) performing base and advance analysis for IS tracking (clonal abundance, clonal expansions and statistics for insertional mutagenesis, etc.), (3) providing basic biology insights of transduced stem cells in vivo.

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**URL** https://calabrialab.github.io/ISAnalytics, https://github.com//calabrialab/isanalytics, https://calabrialab.github.io/ISAnalytics/

**BugReports** https://github.com/calabrialab/ISAnalytics/issues

**biocViews** BiomedicalInformatics, Sequencing, SingleCell

**Depends** R (>= 4.5)

**Imports** utils, dplyr, readr, tidyr, purrr, rlang, tibble, stringr, fs, lubridate, lifecycle, ggplot2, ggrepel, stats, readxl, tools, grDevices, forcats, glue, shiny, shinyWidgets, datamods, bslib, DT

**Encoding** UTF-8

**LazyData** false

**Roxygen** list(markdown = TRUE)

**RoxygenNote** 7.3.2

**Suggests** testthat, covr, knitr, BiocStyle, sessioninfo, rmarkdown, roxygen2, vegan, withr, extraDistr, ggalluvial, scales, gridExtra, R.utils, RefManageR, flexdashboard, circlize, plotly, gtools, eulerr, openxlsx, jsonlite, pheatmap, BiocParallel, progressr, future, doFuture, foreach, psych, data.table, Rcapture

**VignetteBuilder** knitr

**RdMacros** lifecycle

**Config/testthat/edition** 3

**git_url** https://git.bioconductor.org/packages/ISAnalytics

**git_branch** RELEASE_3_21

**git_last_commit** 6c72216

**git_last_commit_date** 2025-04-15

**Repository** Bioconductor 3.21

**Date/Publication** 2025-08-20

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---

`aggregate_metadata` *Performs aggregation on metadata contained in the association file.*

---

## Description

**[Stable]** Groups metadata by the specified grouping keys and returns a summary of info for each group. For more details on how to use this function: `vignette("workflow_start", package = "ISAnalytics")`

## Usage

```
aggregate_metadata(
  association_file,
  grouping_keys = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  aggregating_functions = default_meta_agg(),
  import_stats = lifecycle::deprecated()
)
```

## Arguments

| | |
|---|---|
| `association_file` | The imported association file (via import_association_file) |
| `grouping_keys` | A character vector of column names to form a grouping operation |
| `aggregating_functions` | A data frame containing specifications of the functions to be applied to columns in the association file during aggregation. It defaults to default_meta_agg. The structure of this data frame should be maintained if the user wishes to change the defaults. |
| `import_stats` | **[Deprecated]** The import of VISPA2 stats has been moved to its dedicated function, see import_Vispa2_stats. |

## Value

An aggregated data frame

## See Also

Other Data cleaning and pre-processing: `aggregate_values_by_key()`, `compute_near_integrations()`, `default_meta_agg()`, `outlier_filter()`, `outliers_by_pool_fragments()`, `purity_filter()`, `realign_after_collisions()`, `remove_collisions()`, `threshold_filter()`

## Examples

```
data("association_file", package = "ISAnalytics")
aggreg_meta <- aggregate_metadata(
    association_file = association_file
)
head(aggreg_meta)
```

---

`aggregate_values_by_key` *Aggregates matrices values based on specified key.*

---

## Description

**[Stable]** Performs aggregation on values contained in the integration matrices based on the key and the specified lambda. For more details on how to use this function: `vignette("workflow_start", package = "ISAnalytics")`

## Usage

```
aggregate_values_by_key(
  x,
  association_file,
  value_cols = "Value",
  key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  lambda = list(sum = ~sum(.x, na.rm = TRUE)),
  group = c(mandatory_IS_vars(), annotation_IS_vars()),
  join_af_by = "CompleteAmplificationID"
)
```

## Arguments

| | |
|---|---|
| `x` | A single integration matrix or a list of imported integration matrices |
| `association_file` | The imported association file |
| `value_cols` | A character vector containing the names of the columns to apply the given lambdas. Must be numeric or integer columns. |
| `key` | A string or a character vector with column names of the association file to take as key |
| `lambda` | A named list of functions or purrr-style lambdas. See details section. |
| `group` | Other variables to include in the grouping besides `key`, can be set to NULL |
| `join_af_by` | A character vector representing the joining key between the matrix and the metadata. Useful to re-aggregate already aggregated matrices. |

## Details

**Setting the lambda parameter:**
The lambda parameter should always contain a named list of either functions or purrr-style lambdas. It is also possible to specify the namespace of the function in both ways, for example:

```
lambda = list(sum = sum, desc = psych::describe)
```

Using purrr-style lambdas allows to specify arguments for the functions, keeping in mind that the first parameter should always be `.x`:

```
lambda = list(sum = ~sum(.x, na.rm = TRUE))
```

It is also possible to use custom user-defined functions, keeping in mind that the symbol will be evaluated in the calling environment, for example if the function is called in the global environment and lambda contains "foo" as a function, "foo" will be evaluated in the global environment.

```
foo <- function(x) {
  sum(x)
}

lambda = list(sum = ~sum(.x, na.rm = TRUE), foo = foo)

# Or with lambda notation
lambda = list(sum = ~sum(.x, na.rm = TRUE), foo = ~foo(.x))
```

**Constraints on aggregation functions:**
Functions passed in the lambda parameters must respect a few constraints to properly work and it's the user responsibility to ensure this.

- Functions have to accept as input a numeric or integer vector
- Function should return a single value or a list/data frame: if a list or a data frame is returned as a result, all the columns will be added to the final data frame.

## Value

A list of data frames or a single data frame aggregated according to the specified arguments

## See Also

Other Data cleaning and pre-processing: `aggregate_metadata()`, `compute_near_integrations()`, `default_meta_agg()`, `outlier_filter()`, `outliers_by_pool_fragments()`, `purity_filter()`, `realign_after_collisions()`, `remove_collisions()`, `threshold_filter()`

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
head(aggreg)
```

## annotation_issues *Check for genomic annotation problems in IS matrices.*

### Description

**[Experimental]** This helper function checks if each individual integration site, identified by the `mandatory_IS_vars()`, has been annotated with two or more distinct gene symbols.

### Usage

```
annotation_issues(matrix)
```

### Arguments

| | |
|---|---|
| `matrix` | Either a single matrix or a list of matrices, ideally obtained via `import_parallel_Vispa2Matrices()` or `import_single_Vispa2Matrix()` |

### Value

Either `NULL` if no issues were detected or 1 or more data frames with genomic coordinates of the IS and the number of distinct genes associated

### See Also

Other Import functions helpers: `date_formats()`, `default_af_transform()`, `default_iss_file_prefixes()`, `matching_options()`, `quantification_types()`

### Examples

```
data("integration_matrices", package = "ISAnalytics")
annotation_issues(integration_matrices)
```

## association_file *Example of association file.*

### Description

This file is a simple example of association file. Use it as reference to properly fill out yours. To generate an empty association file to fill see the `generate_blank_association_file()` function.

### Usage

```
data("association_file")
```

### Format

An object of class `data.table` (inherits from `data.frame`) with 53 rows and 83 columns.

## Details

The data was obtained manually by simulating real research data.

## See Also

generate_blank_association_file

---

as_sparse_matrix *Converts tidy integration matrices in the original sparse matrix form.*

---

## Description

**[Stable]** This function is particularly useful when a sparse matrix structure is needed by a specific function (mainly from other packages).

## Usage

```
as_sparse_matrix(
  x,
  single_value_col = "Value",
  fragmentEstimate = "fragmentEstimate",
  seqCount = "seqCount",
  barcodeCount = "barcodeCount",
  cellCount = "cellCount",
  ShsCount = "ShsCount",
  key = pcr_id_column()
)
```

## Arguments

| | |
|---|---|
| x | A single tidy integration matrix or a list of integration matrices. Supports also multi-quantification matrices obtained via comparison_matrix |
| single_value_col | Name of the column containing the values when providing a single-quantification matrix |
| fragmentEstimate | For multi-quantification matrix support: the name of the fragment estimate values column |
| seqCount | For multi-quantification matrix support: the name of the sequence count values column |
| barcodeCount | For multi-quantification matrix support: the name of the barcode count values column |
| cellCount | For multi-quantification matrix support: the name of the cell count values column |
| ShsCount | For multi-quantification matrix support: the name of the Shs Count values column |

key — The name of the sample identifier fields (for aggregated matrices can be a vector with more than 1 element)

## Value

Depending on input, 2 possible outputs:

- A single sparse matrix (data frame) if input is a single quantification matrix
- A list of sparse matrices divided by quantification if input is a single multi-quantification matrix or a list of matrices

## See Also

Other Utilities: `comparison_matrix()`, `enable_progress_bars()`, `export_ISA_settings()`, `generate_Vispa2_launch_AF()`, `generate_blank_association_file()`, `generate_default_folder_structure()`, `import_ISA_settings()`, `separate_quant_matrices()`, `transform_columns()`

## Examples

```
data("integration_matrices", package = "ISAnalytics")
sparse <- as_sparse_matrix(integration_matrices)
```

---

`available_outlier_tests`

*A character vector containing all the names of the currently supported outliers tests that can be called in the function outlier_filter.*

---

## Description

A character vector containing all the names of the currently supported outliers tests that can be called in the function outlier_filter.

## Usage

```
available_outlier_tests()
```

## Value

A character vector

## Examples

```
available_outlier_tests()
```

## available_tags *All available tags for dynamic vars look-up tables.*

### Description

Contains all information associated with critical tags used in the dynamic vars system. To know more see `vignette("workflow_start", package="ISAnalytics")`.

### Usage

```
available_tags()
```

### Value

A data frame

### Examples

```
available_tags()
```

## blood_lineages_default *Default blood lineages info*

### Description

A default table with info relative to different blood lineages associated with cell markers that can be supplied as a parameter to `HSC_population_size_estimate`

### Usage

```
blood_lineages_default()
```

### Value

A data frame

### Examples

```
blood_lineages_default()
```

`circos_genomic_density` *Trace a circos plot of genomic densities.*

## Description

**[Stable]** For this functionality the suggested package circlize is required. Please note that this function is a simple wrapper of basic `circlize` functions, for an in-depth explanation on how the functions work and additional arguments please refer to the official documentation Circular Visualization in R

## Usage

```
circos_genomic_density(
  data,
  gene_labels = NULL,
  label_col = NULL,
  cytoband_specie = "hg19",
  track_colors = "navyblue",
  grDevice = c("png", "pdf", "svg", "jpeg", "bmp", "tiff", "default"),
  file_path = getwd(),
  ...
)
```

## Arguments

| | |
|---|---|
| `data` | Either a single integration matrix or a list of integration matrices. If a list is provided, a separate density track for each data frame is plotted. |
| `gene_labels` | Either `NULL` or a data frame in bed format. See details. |
| `label_col` | Numeric index of the column of `gene_labels` that contains the actual labels. Relevant only if `gene_labels` is not set to `NULL`. |
| `cytoband_specie` | Specie for initializing the cytoband |
| `track_colors` | Colors to give to density tracks. If more than one integration matrix is provided as `data` should be of the same length. Values are recycled if length of `track_colors` is smaller than the length of the input data. |
| `grDevice` | The graphical device where the plot should be traced. `default`, if executing from RStudio is the viewer. |
| `file_path` | If a device other than `default` is chosen, the path on disk where the file should be saved. Defaults to `{current directory}/circos_plot.{device}`. |
| `...` | Additional named arguments to pass on to chosen device, `circlize::circos.par()`, `circlize::circos.genomicDensity()` and `circlize::circos.genomicLabels()` |

## Details

### Providing genomic labels:

If genomic labels should be plotted alongside genomic density tracks, the user should provide them as a simple data frame in standard bed format, namely `chr`, `start`, `end` plus a column containing the labels. NOTE: if the user decides to plot on the default device (viewer in RStudio), he must ensure there is enough space for all elements to be plotted, otherwise an error message is thrown.

## Value

`NULL`

## See Also

Other Plotting functions: `CIS_volcano_plot()`, `HSC_population_plot()`, `fisher_scatterplot()`, `integration_alluvial_plot()`, `sharing_heatmap()`, `sharing_venn()`, `top_abund_tableGrob()`, `top_cis_overtime_heatmap()`

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
by_subj <- aggreg |>
    dplyr::group_by(.data$SubjectID) |>
    dplyr::group_split()
circos_genomic_density(by_subj,
    track_colors = c("navyblue", "gold"),
    grDevice = "default", track.height = 0.1
)
```

---

`CIS_grubbs` *Grubbs test for Common Insertion Sites (CIS).*

---

## Description

**[Stable]** Statistical approach for the validation of common insertion sites significance based on the comparison of the integration frequency at the CIS gene with respect to other genes contained in the surrounding genomic regions. For more details please refer to this paper: https://ashpublications.org/blood/article/117/20/5332/21206/Lentiviral-vector-common-integration-sites-in

## Usage

```
CIS_grubbs(
  x,
  genomic_annotation_file = "hg19",
  grubbs_flanking_gene_bp = 1e+05,
  threshold_alpha = 0.05,
  by = NULL,
  return_missing_as_df = TRUE,
  results_as_list = TRUE
)
```

## Arguments

| | |
|---|---|
| x | An integration matrix, must include the mandatory_IS_vars() columns and the annotation_IS_vars() columns |
| genomic_annotation_file | Database file for gene annotation, see details. |
| grubbs_flanking_gene_bp | Number of base pairs flanking a gene |
| threshold_alpha | Significance threshold |
| by | Either NULL or a character vector of column names. If not NULL, the function will perform calculations for each group and return a list of data frames with the results. E.g. for by = "SubjectID", CIS will be computed for each distinct SubjectID found in the table ("SubjectID" column must be included in the input data frame). |
| return_missing_as_df | Returns those genes present in the input df but not in the refgenes as a data frame? |
| results_as_list | If TRUE return the group computations as a named list, otherwise return a single df with an additional column containing the group id |

## Details

### Genomic annotation file:

A data frame containing genes annotation for the specific genome. From version 1.5.4 the argument genomic_annotation_file accepts only data frames or package provided defaults. The user is responsible for importing the appropriate tabular files if customization is needed. The annotations for the human genome (hg19) and murine genome (mm9) are already included in this package: to use one of them just set the argument genomic_annotation_file to either "hg19" or "mm9". If for any reason the user is performing an analysis on another genome, this file needs to be changed respecting the USCS Genome Browser format, meaning the input file headers should include:

name2, chrom, strand, min_txStart, max_txEnd, minmax_TxLen, average_TxLen, name, min_cdsStart, max_cdsEnd, minmax_CdsLen, average_CdsLen

### Value

A data frame

### Required tags

The function will explicitly check for the presence of these tags:

- chromosome
- locus
- is_strand
- gene_symbol
- gene_strand

### See Also

Other Analysis functions: `HSC_population_size_estimate()`, `compute_abundance()`, `cumulative_is()`, `gene_frequency_fisher()`, `is_sharing()`, `iss_source()`, `sample_statistics()`, `top_integrations()`, `top_targeted_genes()`

### Examples

```
data("integration_matrices", package = "ISAnalytics")
cis <- CIS_grubbs(integration_matrices)
cis
```

---

## CIS_grubbs_overtime *Compute CIS and Grubbs test over different time points and groups.*

---

### Description

**[Experimental]** Computes common insertion sites and Grubbs test for each separate group and separating different time points among the same group. The logic applied is the same as the function `CIS_grubbs()`.

### Usage

```
CIS_grubbs_overtime(
  x,
  genomic_annotation_file = "hg19",
  grubbs_flanking_gene_bp = 1e+05,
  threshold_alpha = 0.05,
  group = "SubjectID",
  timepoint_col = "TimePoint",
  as_df = TRUE,
  return_missing_as_df = TRUE,
  max_workers = NULL
)
```

## Arguments

| | |
|---|---|
| x | An integration matrix, must include the mandatory_IS_vars() columns and the annotation_IS_vars() columns |
| genomic_annotation_file | Database file for gene annotation, see details. |
| grubbs_flanking_gene_bp | Number of base pairs flanking a gene |
| threshold_alpha | Significance threshold |
| group | A character vector of column names that identifies a group. Each group must contain one or more time points. |
| timepoint_col | What is the name of the column containing time points? |
| as_df | Choose the result format: if TRUE the results are returned as a single data frame containing a column for the group id and a column for the time point, if FALSE results are returned in the form of nested lists (one table for each time point and for each group), if "group" results are returned as a list separated for each group but containing a single table with all time points. |
| return_missing_as_df | Returns those genes present in the input df but not in the refgenes as a data frame? |
| max_workers | Maximum number of parallel workers. If NULL the maximum number of workers is calculated automatically. |

## Details

### Genomic annotation file:

A data frame containing genes annotation for the specific genome. From version 1.5.4 the argument genomic_annotation_file accepts only data frames or package provided defaults. The user is responsible for importing the appropriate tabular files if customization is needed. The annotations for the human genome (hg19) and murine genome (mm9) are already included in this package: to use one of them just set the argument genomic_annotation_file to either "hg19" or "mm9". If for any reason the user is performing an analysis on another genome, this file needs to be changed respecting the USCS Genome Browser format, meaning the input file headers should include:

name2, chrom, strand, min_txStart, max_txEnd, minmax_TxLen, average_TxLen, name, min_cdsStart, max_cdsEnd, minmax_CdsLen, average_CdsLen

## Value

A list with results and optionally missing genes info

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
```

```
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
cis_overtime <- CIS_grubbs_overtime(aggreg)
cis_overtime
```

---

`CIS_volcano_plot` *Trace volcano plot for computed CIS data.*

---

## Description

**[Stable]** Traces a volcano plot for IS frequency and CIS results.

## Usage

```
CIS_volcano_plot(
  x,
  onco_db_file = "proto_oncogenes",
  tumor_suppressors_db_file = "tumor_suppressors",
  species = "human",
  known_onco = known_clinical_oncogenes(),
  suspicious_genes = clinical_relevant_suspicious_genes(),
  significance_threshold = 0.05,
  annotation_threshold_ontots = 0.1,
  highlight_genes = NULL,
  title_prefix = NULL,
  return_df = FALSE
)
```

## Arguments

| | |
|---|---|
| `x` | Either a simple integration matrix or a data frame resulting from the call to CIS_grubbs with `add_standard_padjust = TRUE` |
| `onco_db_file` | Uniprot file for proto-oncogenes (see details). If different from default, should be supplied as a path to a file. |
| `tumor_suppressors_db_file` | Uniprot file for tumor-suppressor genes. If different from default, should be supplied as a path to a file. |
| `species` | One between `"human"`, `"mouse"` and `"all"` |
| `known_onco` | Data frame with known oncogenes. See details. |
| `suspicious_genes` | Data frame with clinical relevant suspicious genes. See details. |
| `significance_threshold` | The significance threshold |

annotation_threshold_ontots
: Value above which genes are annotated with colorful labels

highlight_genes
: Either NULL or a character vector of genes to be highlighted in the plot even if they're not above the threshold

title_prefix
: A string or character vector to be displayed in the title - usually the project name and other characterizing info. If a vector is supplied, it is concatenated in a single string via paste()

return_df
: Return the data frame used to generate the plot? This can be useful if the user wants to manually modify the plot with ggplot2. If TRUE the function returns a list containing both the plot and the data frame.

## Details

### Input data frame:

Users can supply as x either a simple integration matrix or a data frame resulting from the call to CIS_grubbs. In the first case an internal call to the function CIS_grubbs() is performed.

### Oncogene and tumor suppressor genes files:

These files are included in the package for user convenience and are simply UniProt files with gene annotations for human and mouse. For more details on how this files were generated use the help ?tumor_suppressors, ?proto_oncogenes

### Known oncogenes:

The default values are included in this package and it can be accessed by doing:

```
known_clinical_oncogenes()
```

If the user wants to change this parameter the input data frame must preserve the column structure. The same goes for the suspicious_genes parameter (DOIReference column is optional):

```
clinical_relevant_suspicious_genes()
```

## Value

A plot or a list containing a plot and a data frame

## Required tags

The function will explicitly check for the presence of these tags:

- gene_symbol

## See Also

Other Plotting functions: HSC_population_plot(), circos_genomic_density(), fisher_scatterplot(), integration_alluvial_plot(), sharing_heatmap(), sharing_venn(), top_abund_tableGrob(), top_cis_overtime_heatmap()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
cis_plot <- CIS_volcano_plot(integration_matrices,
    title_prefix = "PJ01"
)
cis_plot
```

---

`clinical_relevant_suspicious_genes` *Clinical relevant suspicious genes (for mouse and human).*

---

## Description

Clinical relevant suspicious genes (for mouse and human).

## Usage

```
clinical_relevant_suspicious_genes()
```

## Value

A data frame

## See Also

Other Plotting function helpers: `known_clinical_oncogenes()`

## Examples

```
clinical_relevant_suspicious_genes()
```

---

`comparison_matrix` *Obtain a single integration matrix from individual quantification matrices.*

---

## Description

**[Stable]** Takes a list of integration matrices referring to different quantification types and merges them into a single data frame with multiple value columns, each renamed according to their quantification type of reference.

## Usage

```
comparison_matrix(
  x,
  fragmentEstimate = "fragmentEstimate",
  seqCount = "seqCount",
  barcodeCount = "barcodeCount",
  cellCount = "cellCount",
  ShsCount = "ShsCount",
  value_col_name = "Value"
)
```

## Arguments

| | |
|---|---|
| x | A named list of integration matrices, ideally obtained via import_parallel_Vispa2Matrices. Names must be quantification types in `quantification_types()`. |
| fragmentEstimate | The name of the output column for fragment estimate values |
| seqCount | The name of the output column for sequence count values |
| barcodeCount | The name of the output column for barcode count values |
| cellCount | The name of the output column for cell count values |
| ShsCount | The name of the output column for Shs count values |
| value_col_name | Name of the column containing the corresponding values in the single matrices |

## Value

A single data frame

## See Also

quantification_types

Other Utilities: `as_sparse_matrix()`, `enable_progress_bars()`, `export_ISA_settings()`, `generate_Vispa2_launch_`
`generate_blank_association_file()`, `generate_default_folder_structure()`, `import_ISA_settings()`,
`separate_quant_matrices()`, `transform_columns()`

## Examples

```
sc <- tibble::tribble(
    ~chr, ~integration_locus, ~strand, ~CompleteAmplificationID, ~Value,
    "1", 45324, "+", "ID1", 543,
    "2", 52423, "-", "ID1", 42,
    "6", 54623, "-", "ID2", 67,
    "X", 12314, "+", "ID3", 8
)
fe <- tibble::tribble(
    ~chr, ~integration_locus, ~strand, ~CompleteAmplificationID, ~Value,
    "1", 45324, "+", "ID1", 56.76,
    "2", 52423, "-", "ID1", 78.32,
    "6", 54623, "-", "ID2", 123.45,
```

```
    "X", 12314, "+", "ID3", 5.34
)
comparison_matrix(list(
    fragmentEstimate = fe,
    seqCount = sc
))
```

---

compute_abundance *Computes the abundance for every integration event in the input data frame.*

---

## Description

**[Stable]** Abundance is obtained for every integration event by calculating the ratio between the single value and the total value for the given group.

## Usage

```
compute_abundance(
  x,
  columns = c("fragmentEstimate_sum"),
  percentage = TRUE,
  key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  keep_totals = FALSE
)
```

## Arguments

| | |
|---|---|
| x | An integration matrix - aka a data frame that includes the `mandatory_IS_vars()` as columns. The matrix can either be aggregated (via `aggregate_values_by_key()`) or not. |
| columns | A character vector of column names to process, must be numeric or integer columns |
| percentage | Add abundance as percentage? |
| key | The key to group by when calculating totals |
| keep_totals | A value between `TRUE`, `FALSE` or `df`. If `TRUE`, the intermediate totals for each group will be kept in the output data frame as a dedicated column with a trailing "_tot". If `FALSE`, totals won't be included in the output data frame. If `df`, the totals are returned to the user as a separate data frame, together with the abundance data frame. |

## Details

Abundance will be computed upon the user selected columns in the `columns` parameter. For each column a corresponding relative abundance column (and optionally a percentage abundance column) will be produced.

### Value

Either a single data frame with computed abundance values or a list of 2 data frames (abundance_df, quant_totals)

### Required tags

The function will explicitly check for the presence of these tags:

- All columns declared in `mandatory_IS_vars()`

### See Also

Other Analysis functions: `CIS_grubbs()`, `HSC_population_size_estimate()`, `cumulative_is()`, `gene_frequency_fisher()`, `is_sharing()`, `iss_source()`, `sample_statistics()`, `top_integrations()`, `top_targeted_genes()`

### Examples

```
data("integration_matrices", package = "ISAnalytics")
abund <- compute_abundance(
    x = integration_matrices,
    columns = "fragmentEstimate",
    key = "CompleteAmplificationID"
)
head(abund)
```

---

## compute_near_integrations

*Scans input matrix to find and merge near integration sites.*

---

### Description

**[Stable]** This function scans the input integration matrix to detect eventual integration sites that are too "near" to each other and merges them into single integration sites adjusting their values if needed.

### Usage

```
compute_near_integrations(
  x,
  threshold = 4,
  is_identity_tags = c("chromosome", "is_strand"),
  keep_criteria = c("max_value", "keep_first"),
  value_columns = c("seqCount", "fragmentEstimate"),
  max_value_column = "seqCount",
  sample_id_column = pcr_id_column(),
  additional_agg_lambda = list(.default = default_rec_agg_lambdas()),
  max_workers = 4,
```

```
  map_as_file = TRUE,
  file_path = default_report_path(),
  strand_specific = lifecycle::deprecated()
)
```

## Arguments

| | |
|---|---|
| x | An integration matrix |
| threshold | A single integer that represents an absolute number of bases for which two integrations are considered distinct. If the threshold is set to 3 it means, provided fields chr and strand are the same, integrations sites which have at least 3 bases in between them are considered distinct. |
| is_identity_tags | Character vector of tags that identify the integration event as distinct (except for "locus"). See details. |
| keep_criteria | While scanning, which integration should be kept? The 2 possible choices for this parameter are:<br>• "max_value": keep the integration site which has the highest value (and collapse other values on that integration).<br>• "keep_first": keeps the first integration |
| value_columns | Character vector, contains the names of the numeric experimental columns |
| max_value_column | The column that has to be considered for searching the maximum value |
| sample_id_column | The name of the column containing the sample identifier |
| additional_agg_lambda | A named list containing aggregating functions for additional columns. See details. |
| max_workers | Maximum parallel workers allowed |
| map_as_file | Produce recalibration map as a .tsv file? |
| file_path | String representing the path were the file will be saved. Must be a folder. Relevant only if map_as_file is TRUE. |
| strand_specific | **[Deprecated]** Deprecated, use is_identity_tags |

## Details

**The concept of "near":**

An integration event is uniquely identified by all fields specified in the mandatory_IS_vars() look-up table. It can happen to find IS that are formally distinct (different combination of values in the fields), but that should not considered distinct in practice, since they represent the same integration event - this may be due to artefacts at the putative locus of the IS in the merging of multiple sequencing libraries.

We say that an integration event IS1 is near to another integration event IS2 if the absolute difference of their loci is strictly lower than the set threshold.

**The IS identity:**

There is also another aspect to be considered. Since the algorithm is based on a sliding window mechanism, on which groups of IS should we set and slide the window?

By default, we have 3 fields in the `mandatory_IS_vars()`: chr, integration_locus, strand, and we assume that all the fields contribute to the identity of the IS. This means that IS1 and IS2 can be compared only if they have the same chromosome and the same strand. However, if we would like to exclude the strand of the integration from our considerations then IS1 and IS2 can be selected from all the events that fall on the same chromosome. A practical example:

IS1 = `(chr = "1", strand = "+", integration_locus = 14568)`

IS2 = `(chr = "1", strand = "-", integration_locus = 14567)`

if `is_identity_tags = c("chromosome", "is_strand")` IS1 and IS2 are considered distinct because they differ in strand, therefore no correction will be applied to loci of either of the 2. If `is_identity_tags = c("chromosome")` then IS1 and IS2 are considered near, because the strand is irrelevant, hence one of the 2 IS will change locus.

**Aggregating near IS:**

IS that fall in the same interval are evaluated according to the criterion selected - if recalibration is necessary, rows with the same sample ID are aggregated in a single row with a quantification value that is the sum of all the merged rows.

If the input integration matrix contains annotation columns, that is additional columns that are not

- part of the mandatory IS vars (see `mandatory_IS_vars()`)
- part of the annotation IS vars (see `annotation_IS_vars()`)
- the sample identifier column
- the quantification column

it is possible to specify how they should be aggregated. Defaults are provided for each column type (character, integer, numeric...), but custom functions can be specified as a named list, where names are column names in `x` and values are functions to be applied. NOTE: functions must be purrr-style lambdas and they must perform some kind of aggregating operation, aka they must take a vector as input and return a single value. The type of the output should match the type of the target column. If you specify custom lambdas, provide defaults in the special element `.defaults`. Example:

```
list(
  numeric_col = ~ sum(.x),
  char_col = ~ paste0(.x, collapse = ", "),
  .defaults = default_rec_agg_lambdas()
)
```

## Value

An integration matrix with same or less number of rows

## Required tags

The function will explicitly check for the presence of these tags:

- chromosome

- locus
- is_strand
- gene_symbol

## Note

We do recommend to use this function in combination with comparison_matrix to automatically perform re-calibration on all quantification matrices.

## See Also

Other Data cleaning and pre-processing: aggregate_metadata(), aggregate_values_by_key(), default_meta_agg(), outlier_filter(), outliers_by_pool_fragments(), purity_filter(), realign_after_collisions(), remove_collisions(), threshold_filter()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
rec <- compute_near_integrations(
    x = integration_matrices, map_as_file = FALSE
)
head(rec)
```

---

# cumulative_count_union

*Integrations cumulative count in time by sample*

---

## Description

**[Defunct]** This function was deprecated in favour of a single function, please use cumulative_is instead.

## Usage

```
cumulative_count_union(
  x,
  association_file = NULL,
  timepoint_column = "TimePoint",
  key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  include_tp_zero = FALSE,
  zero = "0000",
  aggregate = FALSE,
  ...
)
```

### Arguments

| | |
|---|---|
| x | A simple integration matrix or an aggregated matrix (see details) |
| association_file | NULL or the association file for x if aggregate is set to TRUE |
| timepoint_column | What is the name of the time point column? |
| key | The aggregation key - must always contain the timepoint_column |
| include_tp_zero | Include timepoint 0? |
| zero | How is 0 coded in the data frame? |
| aggregate | Should x be aggregated? |
| ... | Additional parameters to pass to aggregate_values_by_key |

### Value

A data frame

### Examples

```
## Not run:
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
cumulative_count <- cumulative_count_union(aggreg)
cumulative_count

## End(Not run)
```

---

## cumulative_is *Expands integration matrix with the cumulative IS union over time.*

---

### Description

**[Experimental]** Given an input integration matrix that can be grouped over time, this function adds integrations in groups assuming that if an integration is observed at time point "t" then it is also observed in time point "t+1".

## Usage

```
cumulative_is(
  x,
  key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  timepoint_col = "TimePoint",
  include_tp_zero = FALSE,
  counts = TRUE,
  keep_og_is = FALSE,
  expand = TRUE
)
```

## Arguments

| | |
|---|---|
| x | An integration matrix, ideally aggregated via aggregate_values_by_key() |
| key | The aggregation key used |
| timepoint_col | The name of the time point column |
| include_tp_zero | Should time point 0 be included? |
| counts | Add cumulative counts? Logical |
| keep_og_is | Keep original set of integrations as a separate column? |
| expand | If FALSE, for each group, the set of integration sites is returned in a separate column as a nested table, otherwise the resulting column is unnested. |

## Value

A data frame

## Required tags

The function will explicitly check for the presence of these tags:

- All columns declared in mandatory_IS_vars()
- Checks if the matrix is annotated by assessing presence of annotation_IS_vars()

## See Also

Other Analysis functions: CIS_grubbs(), HSC_population_size_estimate(), compute_abundance(), gene_frequency_fisher(), is_sharing(), iss_source(), sample_statistics(), top_integrations(), top_targeted_genes()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
```

```
)
cumulated_is <- cumulative_is(aggreg)
cumulated_is
```

---

`date_formats` *Possible choices for the* `dates_format` *parameter in* `import_association_file`*,* `import_parallel_vispa2Matrices_interactive` *and* `import_parallel_vispa2Matrices_auto`*.*

---

## Description

All options correspond to `lubridate` functions, see more in the dedicated package documentation.

## Usage

```
date_formats()
```

## Value

A character vector

## See Also

`import_association_file`, `import_parallel_Vispa2Matrices_auto`

Other Import functions helpers: `annotation_issues()`, `default_af_transform()`, `default_iss_file_prefixes()`, `matching_options()`, `quantification_types()`

## Examples

```
date_formats()
```

---

`default_af_transform` *Default transformations to apply to association file columns.*

---

## Description

A list of default transformations to apply to the association file columns after importing it via `import_association_file()`

## Usage

```
default_af_transform(convert_tp)
```

## Arguments

| | |
|---|---|
| `convert_tp` | The value of the argument `convert_tp` in the call to `import_association_file()` |

## Value

A named list of lambdas

## See Also

Other Import functions helpers: annotation_issues(), date_formats(), default_iss_file_prefixes(), matching_options(), quantification_types()

## Examples

```
default_af_transform(TRUE)
```

---

# default_iss_file_prefixes

*Default regex prefixes for Vispa2 stats files.*

---

## Description

Note that each element is a regular expression.

## Usage

```
default_iss_file_prefixes()
```

## Value

A character vector of regexes

## See Also

Other Import functions helpers: annotation_issues(), date_formats(), default_af_transform(), matching_options(), quantification_types()

## Examples

```
default_iss_file_prefixes()
```

default_meta_agg *Default metadata aggregation function table*

## Description

A default columns-function specifications for aggregate_metadata

## Usage

```
default_meta_agg()
```

## Details

This data frame contains four columns:

- `Column`: holds the name of the column in the association file that should be processed
- `Function`: contains either the name of a function (e.g. mean) or a purrr-style lambda (e.g. `~ mean(.x, na.rm = TRUE)`). This function will be applied to the corresponding column specified in `Column`
- `Args`: optional additional arguments to pass to the corresponding function. This is relevant ONLY if the corresponding `Function` is a simple function and not a purrr-style lambda.
- `Output_colname`: a `glue` specification that will be used to determine a unique output column name. See glue for more details.

## Value

A data frame

## See Also

Other Data cleaning and pre-processing: `aggregate_metadata()`, `aggregate_values_by_key()`, `compute_near_integrations()`, `outlier_filter()`, `outliers_by_pool_fragments()`, `purity_filter()`, `realign_after_collisions()`, `remove_collisions()`, `threshold_filter()`

## Examples

```
default_meta_agg()
```

default_rec_agg_lambdas *Defaults for column aggregations in* `compute_near_integrations()`.

## Description

Defaults for column aggregations in `compute_near_integrations()`.

## Usage

```
default_rec_agg_lambdas()
```

## Value

A named list of lambdas

## Examples

```
default_rec_agg_lambdas()
```

default_report_path *Default folder for saving ISAnalytics reports. Supplied as default argument for several functions.*

## Description

Default folder for saving ISAnalytics reports. Supplied as default argument for several functions.

## Usage

```
default_report_path()
```

## Value

A path

## Examples

```
default_report_path()
```

## default_stats *A set of pre-defined functions for* sample_statistics.

### Description

A set of pre-defined functions for sample_statistics.

### Usage

```
default_stats()
```

### Value

A named list of functions/purrr-style lambdas

### Examples

```
default_stats()
```

## enable_progress_bars *Enable global progress bars for ISAnalytics functions.*

### Description

This is a simple wrapper around functions from the package progressr. To customize the appearance of the progress bar, please refer to progressr documentation.

### Usage

```
enable_progress_bars()
```

### Value

NULL

### See Also

Other Utilities: as_sparse_matrix(), comparison_matrix(), export_ISA_settings(), generate_Vispa2_launch_AF(
generate_blank_association_file(), generate_default_folder_structure(), import_ISA_settings(),
separate_quant_matrices(), transform_columns()

### Examples

```
enable_progress_bars()
progressr::handlers(global = FALSE) # Deactivate
```

## export_ISA_settings *Export a dynamic vars settings profile.*

### Description

This function allows exporting the currently set dynamic vars in json format so it can be quickly imported later. Dynamic variables need to be properly set via the setter functions before calling the function. For more details, refer to the dedicated vignette `vignette("workflow_start", package="ISAnalytics")`.

### Usage

```
export_ISA_settings(folder, setting_profile_name)
```

### Arguments

| | |
|---|---|
| `folder` | The path to the folder where the file should be saved. If the folder doesn't exist, it gets created automatically |
| `setting_profile_name` | A name for the settings profile |

### Value

`NULL`

### See Also

Other Utilities: `as_sparse_matrix()`, `comparison_matrix()`, `enable_progress_bars()`, `generate_Vispa2_launch_AF`
`generate_blank_association_file()`, `generate_default_folder_structure()`, `import_ISA_settings()`,
`separate_quant_matrices()`, `transform_columns()`

### Examples

```
tmp_folder <- tempdir()
export_ISA_settings(tmp_folder, "DEFAULT")
```

## fisher_scatterplot *Plot results of gene frequency Fisher's exact test.*

### Description

**[Stable]** Plots results of Fisher's exact test on gene frequency obtained via `gene_frequency_fisher()` as a scatterplot.

## Usage

```
fisher_scatterplot(
  fisher_df,
  p_value_col = "Fisher_p_value_fdr",
  annot_threshold = 0.05,
  annot_color = "red",
  gene_sym_col = "GeneName",
  do_not_highlight = NULL,
  keep_not_highlighted = TRUE
)
```

## Arguments

| | |
|---|---|
| fisher_df | Test results obtained via gene_frequency_fisher() |
| p_value_col | Name of the column containing the p-value to consider |
| annot_threshold | Annotate with a different color if a point is below the significance threshold. Single numerical value. |
| annot_color | The color in which points below the threshold should be annotated |
| gene_sym_col | The name of the column containing the gene symbol |
| do_not_highlight | Either NULL, a character vector, an expression or a purrr-style lambda. Tells the function to ignore the highlighting and labeling of these genes even if their p-value is below the threshold. See details. |
| keep_not_highlighted | If present, how should not highlighted genes be treated? If set to TRUE points are plotted and colored with the chosen color scale. If set to FALSE the points are removed entirely from the plot. |

## Details

### Specifying genes to avoid highlighting:

In some cases, users might want to avoid highlighting certain genes even if their p-value is below the threshold. To do so, use the argument do_not_highlight: character vectors are appropriate for specific genes that are to be excluded, expressions or lambdas allow a finer control. For example we can supply:

```
expr <- rlang::expr(!stringr::str_starts(GeneName, "MIR") &
                      average_TxLen_1 >= 300)
```

with this expression, genes that have a p-value < threshold and start with "MIR" or have an average_TxLen_1 lower than 300 are excluded from the highlighted points. NOTE: keep in mind that expressions are evaluated inside a dplyr::filter context.

Similarly, lambdas are passed to the filtering function but only operate on the column containing the gene symbol.

```
lambda <- ~ stringr::str_starts(.x, "MIR")
```

## Value

A plot

## See Also

Other Plotting functions: CIS_volcano_plot(), HSC_population_plot(), circos_genomic_density(), integration_alluvial_plot(), sharing_heatmap(), sharing_venn(), top_abund_tableGrob(), top_cis_overtime_heatmap()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
cis <- CIS_grubbs(aggreg, by = "SubjectID")
fisher <- gene_frequency_fisher(cis$cis$PT001, cis$cis$PT002,
    min_is_per_gene = 2
)
fisher_scatterplot(fisher)
```

---

# generate_blank_association_file

*Create a blank association file.*

---

## Description

Produces a blank association file to start using both VISPA2 and ISAnalytics

## Usage

```
generate_blank_association_file(path)
```

## Arguments

| | |
|---|---|
| path | The path on disk where the file should be written - must be a file |

## Value

NULL

## See Also

Other Utilities: as_sparse_matrix(), comparison_matrix(), enable_progress_bars(), export_ISA_settings(), generate_Vispa2_launch_AF(), generate_default_folder_structure(), import_ISA_settings(), separate_quant_matrices(), transform_columns()

## Examples

```
temp <- tempfile()
generate_blank_association_file(temp)
```

---

`generate_default_folder_structure` *Generate a default folder structure, following VISPA2 standards*

---

## Description

The function produces a folder structure in the file system at the provided path that respects VISPA2 standards, with package-included data.

## Usage

```
generate_default_folder_structure(
  type = "correct",
  dir = tempdir(),
  af = "default",
  matrices = "default"
)
```

## Arguments

| | |
|---|---|
| `type` | One value between `"correct"`, `"incorrect"` and `"both"`. Tells the function wheter to produce a correct structure or introduce some errors (mainly for testing purposes). |
| `dir` | Path to the folder in which the structure will be produced |
| `af` | Either `"default"` for the association file provided as example in the package or a custom association file as a data frame |
| `matrices` | Either `"default"` for integration matrices provided as example in the package or a custom multi-quantification matrix |

## Value

A named list containing the path to the association file and the path to the top level folder(s) of the structure

## Required tags

The function will explicitly check for the presence of these tags:

- project_id
- tag_seq
- vispa_concatenate

## See Also

Other Utilities: as_sparse_matrix(), comparison_matrix(), enable_progress_bars(), export_ISA_settings(), generate_Vispa2_launch_AF(), generate_blank_association_file(), import_ISA_settings(), separate_quant_matrices(), transform_columns()

## Examples

```
fs_path <- generate_default_folder_structure(type = "correct")
fs_path
```

---

# generate_Vispa2_launch_AF

*Creates a reduced association file for a VISPA2 run, given project and pool*

---

## Description

The function selects the appropriate columns and prepares a file for the launch of VISPA2 pipeline for each project/pool pair specified.

## Usage

```
generate_Vispa2_launch_AF(association_file, project, pool, path)
```

## Arguments

| | |
|---|---|
| association_file | The imported association file (via import_association_file()) |
| project | A vector of characters containing project names |
| pool | A vector of characters containing pool names |
| path | A single string representing the path to the folder where files should be written. If the folder doesn't exist it will be created. |

## Details

Note: the function is vectorized, meaning you can specify more than one project and more than one pool as vectors of characters, but you must ensure that:

- Both project and pool vectors have the same length
- You correclty type names in corresponding positions, for example c("PJ01", "PJ01") - c("POOL01", "POOL02"). If you type a pool in the position of a corresponding project that doesn't match no file will be produced since that pool doesn't exist in the corresponding project.

## Value

NULL

### Required tags

The function will explicitly check for the presence of these tags:

- cell_marker
- fusion_id
- pcr_repl_id
- pool_id
- project_id
- subject
- tag_id
- tissue
- tp_days
- vector_id

The names of the pools in the `pool` argument is checked against the column corresponding to the `pool_id` tag.

### See Also

Other Utilities: `as_sparse_matrix()`, `comparison_matrix()`, `enable_progress_bars()`, `export_ISA_settings()`, `generate_blank_association_file()`, `generate_default_folder_structure()`, `import_ISA_settings()`, `separate_quant_matrices()`, `transform_columns()`

### Examples

```
temp <- tempdir()
data("association_file", package = "ISAnalytics")
generate_Vispa2_launch_AF(association_file, "PJ01", "POOL01", temp)
```

---

## gene_frequency_fisher *Compute Fisher's exact test on gene frequencies.*

---

### Description

**[Experimental]** Provided 2 data frames with calculations for CIS, via `CIS_grubbs()`, computes Fisher's exact test. Results can be plotted via `fisher_scatterplot()`.

### Usage

```
gene_frequency_fisher(
  cis_x,
  cis_y,
  min_is_per_gene = 3,
  gene_set_method = c("intersection", "union"),
  onco_db_file = "proto_oncogenes",
```

```
  tumor_suppressors_db_file = "tumor_suppressors",
  species = "human",
  known_onco = known_clinical_oncogenes(),
  suspicious_genes = clinical_relevant_suspicious_genes(),
  significance_threshold = 0.05,
  remove_unbalanced_0 = TRUE
)
```

## Arguments

| | |
|---|---|
| cis_x | A data frame obtained via CIS_grubbs() |
| cis_y | A data frame obtained via CIS_grubbs() |
| min_is_per_gene | Used for pre-filtering purposes. Genes with a number of distinct integration less than this number will be filtered out prior calculations. Single numeric or integer. |
| gene_set_method | One between "intersection" and "union". When merging the 2 data frames, intersection will perform an inner join operation, while union will perform a full join operation. |
| onco_db_file | Uniprot file for proto-oncogenes (see details). If different from default, should be supplied as a path to a file. |
| tumor_suppressors_db_file | Uniprot file for tumor-suppressor genes. If different from default, should be supplied as a path to a file. |
| species | One between "human", "mouse" and "all" |
| known_onco | Data frame with known oncogenes. See details. |
| suspicious_genes | Data frame with clinical relevant suspicious genes. See details. |
| significance_threshold | Significance threshold for the Fisher's test p-value |
| remove_unbalanced_0 | Remove from the final output those pairs in which there are no IS for one group or the other and the number of IS of the non-missing group are less than the mean number of IS for that group |

## Details

### Oncogene and tumor suppressor genes files:

These files are included in the package for user convenience and are simply UniProt files with gene annotations for human and mouse. For more details on how this files were generated use the help ?tumor_suppressors, ?proto_oncogenes

### Known oncogenes:

The default values are included in this package and it can be accessed by doing:

```
known_clinical_oncogenes()
```

If the user wants to change this parameter the input data frame must preserve the column structure. The same goes for the `suspicious_genes` parameter (DOIReference column is optional):

```
clinical_relevant_suspicious_genes()
```

## Value

A data frame

## Required tags

The function will explicitly check for the presence of these tags:

- gene_symbol

## See Also

Other Analysis functions: `CIS_grubbs()`, `HSC_population_size_estimate()`, `compute_abundance()`, `cumulative_is()`, `is_sharing()`, `iss_source()`, `sample_statistics()`, `top_integrations()`, `top_targeted_genes()`

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
cis <- CIS_grubbs(aggreg, by = "SubjectID")
fisher <- gene_frequency_fisher(cis$cis$PT001, cis$cis$PT002,
    min_is_per_gene = 2
)
fisher
```

---

# HSC_population_plot *Plot of the estimated HSC population size for each patient.*

---

## Description

Plot of the estimated HSC population size for each patient.

## Usage

```
HSC_population_plot(
  estimates,
  project_name,
  timepoints = "Consecutive",
  models = "Mth Chao (LB)"
)
```

## Arguments

| | |
|---|---|
| estimates | The estimates data frame, obtained via HSC_population_size_estimate |
| project_name | The project name, will be included in the plot title |
| timepoints | Which time points to plot? One between "All", "Stable" and "Consecutive" |
| models | Name of the models to plot (as they appear in the column of the estimates) |

## Value

A plot

## See Also

Other Plotting functions: CIS_volcano_plot(), circos_genomic_density(), fisher_scatterplot(), integration_alluvial_plot(), sharing_heatmap(), sharing_venn(), top_abund_tableGrob(), top_cis_overtime_heatmap()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
aggreg_meta <- aggregate_metadata(
    association_file = association_file
)
estimate <- HSC_population_size_estimate(
    x = aggreg,
    metadata = aggreg_meta,
    stable_timepoints = c(90, 180, 360),
    cell_type = "Other"
)
p <- HSC_population_plot(estimate$est, "PJ01")
p
```

`HSC_population_size_estimate` *Hematopoietic stem cells population size estimate.*

## Description

**[Stable]** Hematopoietic stem cells population size estimate with capture-recapture models.

## Usage

```
HSC_population_size_estimate(
  x,
  metadata,
  stable_timepoints = NULL,
  aggregation_key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  blood_lineages = blood_lineages_default(),
  timepoint_column = "TimePoint",
  seqCount_column = "seqCount_sum",
  fragmentEstimate_column = "fragmentEstimate_sum",
  seqCount_threshold = 3,
  fragmentEstimate_threshold = 3,
  nIS_threshold = 5,
  cell_type = "MYELOID",
  tissue_type = "PB",
  max_workers = 4
)
```

## Arguments

| | |
|---|---|
| `x` | An aggregated integration matrix. See details. |
| `metadata` | An aggregated association file. See details. |
| `stable_timepoints` | A numeric vector or NULL if there are no stable time points. NOTE: the vector is NOT intended as a sequence min-max, every stable time point has to be specified individually |
| `aggregation_key` | A character vector indicating the key used for aggregating x and metadata. Note that x and metadata should always be aggregated with the same key. |
| `blood_lineages` | A data frame containing information on the blood lineages. Users can supply their own, provided the columns `CellMarker` and `CellType` are present. |
| `timepoint_column` | What is the name of the time point column to use? Note that this column must be present in the key. |
| `seqCount_column` | What is the name of the column in x containing the values of sequence count quantification? |

`fragmentEstimate_column`
: What is the name of the column in x containing the values of fragment estimate quantification? If fragment estimate is not present in the matrix, param should be set to `NULL`.

`seqCount_threshold`
: A single numeric value. After re-aggregating `x`, rows with a value greater or equal will be kept, the others will be discarded.

`fragmentEstimate_threshold`
: A single numeric value. Threshold value for fragment estimate, see details.

`nIS_threshold`
: A single numeric value. If a group (row) in the metadata data frame has a count of distinct integration sites strictly greater than this number it will be kept, otherwise discarded.

`cell_type`
: The cell types to include in the models. Note that the matching is case-insensitive.

`tissue_type`
: The tissue types to include in the models. Note that the matching is case-insensitive.

`max_workers`
: Maximum parallel workers allowed

## Value

A data frame with the results of the estimates

## Input formats

Both `x` and `metadata` should be supplied to the function in aggregated format (ideally through the use of `aggregate_metadata` and `aggregate_values_by_key`). Note that the `aggregation_key`, aka the vector of column names used for aggregation, must contain at least the columns associated with the tags `subject`, `cell_marker`, `tissue` and a time point column (the user can specify the name of the column in the argument `timepoint_column`).

## Specifying more than one group

Groups for the estimates are computed as a pair of cell type and tissue. If the user wishes to compute estimates for more than one combination of cell type and tissue, it is possible to specify them as character vectors to the fields `cell_type` and `tissue_type` respectively, noting that:

- Vectors must have the same length or one of the 2 has to be of length 1
- It is a responsibility of the user to check whether the combination exists in the dataset provided.

Example:

```
estimate <- HSC_population_size_estimate(
    x = aggreg,
    metadata = aggreg_meta,
    cell_type = c("MYELOID", "T", "B"),
    tissue_type = "PB"
)

# Evaluated groups will be:
```

```
# - MYELOID PB
# - T PB
# - B PB
```

Note that estimates are computed individually for each group.

### On time points

If `stable_timepoints` is a vector with length > 1, the function will look for the first available stable time point and slice the data from that time point onward. If `NULL` is supplied instead, it means there are no stable time points available. Note that 0 time points are ALWAYS discarded. Also, to be included in the analysis, a group must have at least 2 distinct non-zero time points. NOTE: the vector passed has to contain all individual time points, not just the minimum and maximum

### Setting a threshold for fragment estimate

If fragment estimate is present in the input matrix, the filtering logic changes slightly: rows in the original matrix are kept if the sequence count value is greater or equal than the `seqCount_threshold` AND the fragment estimate value is greater or equal to the `fragmentEstimate_threshold` IF PRESENT (non-zero value). This means that for rows that miss fragment estimate, the filtering logic will be applied only on sequence count. If the user wishes not to use the combined filtering with fragment estimate, simply set `fragmentEstimate_threshold = 0`.

### Required tags

The function will explicitly check for the presence of these tags:

- subject
- tissue
- cell_marker

### See Also

Other Analysis functions: `CIS_grubbs()`, `compute_abundance()`, `cumulative_is()`, `gene_frequency_fisher()`, `is_sharing()`, `iss_source()`, `sample_statistics()`, `top_integrations()`, `top_targeted_genes()`

### Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
aggreg_meta <- aggregate_metadata(association_file = association_file)
estimate <- HSC_population_size_estimate(
    x = aggreg,
    metadata = aggreg_meta,
    fragmentEstimate_column = NULL,
```

```
    stable_timepoints = c(90, 180, 360),
    cell_type = "Other"
)
```

---

`import_association_file` *Import the association file from disk*

---

## Description

**[Stable]** Imports the association file and optionally performs a check on the file system starting from the root to assess the alignment between the two.

## Usage

```
import_association_file(
  path,
  root = NULL,
  dates_format = "ymd",
  separator = "\t",
  filter_for = NULL,
  import_iss = FALSE,
  convert_tp = TRUE,
  report_path = default_report_path(),
  transformations = default_af_transform(convert_tp),
  tp_padding = lifecycle::deprecated(),
  ...
)
```

## Arguments

| | |
|---|---|
| `path` | The path on disk to the association file. |
| `root` | The path on disk of the root folder of VISPA2 output or `NULL`. See details. |
| `dates_format` | A single string indicating how dates should be parsed. Must be a value in: `date_formats()` |
| `separator` | The column separator used in the file |
| `filter_for` | A named list where names represent column names that must be filtered. For example: `list(ProjectID = c("PROJECT1", "PROJECT2))` will filter the association file so that it contains only those rows for which the value of the column "ProjectID" is one of the specified values. If multiple columns are present in the list all filtering conditions are applied as a logical AND. |
| `import_iss` | Import VISPA2 pool stats and merge them with the association file? Logical value |
| `convert_tp` | Should be time points be converted into months and years? Logical value |

| | |
|---|---|
| report_path | The path where the report file should be saved. Can be a folder or NULL if no report should be produced. Defaults to {user_home}/ISAnalytics_reports. |
| transformations | Either NULL or a named list of purrr-style lambdas where names are column names the function should be applied to. |
| tp_padding | **[Deprecated]** Deprecated. Use transformations instead. |
| ... | Additional arguments to pass to import_Vispa2_stats |

## Details

### Transformations:

Lambdas provided in input in the transformations argument, must be transformations, aka functions that take in input a vector and return a vector of the same length as the input.

If the transformation list contains column names that are not present in the data frame, they are simply ignored.

### File system alignment:

If the root argument is set to NULL no file system alignment is performed. This allows to import the basic file but it won't be possible to perform automated matrix and stats import. For more details see the "How to use import functions" vignette: vignette("workflow_start", package = "ISAnalytics")

### Time point conversion:

The time point conversion is based on the following logic, given TPD is the column containing the time point expressed in days and TPM and TPY are respectively the time points expressed as month and years

- If TPD is NA –> NA (for both months and years)
- TPM = 0, TPY = 0 if and only if TPD = 0

For conversion in months:

- TPM = ceiling(TPD/30) if TPD < 30 otherwise TPM = round(TPD/30)

For conversion in years:

- TPY = ceiling(TPD/360)

## Value

The data frame containing metadata

## Required tags

The function will explicitly check for the presence of these tags:

- project_id
- pool_id
- tag_seq
- subject
- tissue

- tp_days
- cell_marker
- pcr_replicate
- vispa_concatenate
- pcr_repl_id
- proj_folder

The function will use all the available specifications contained in `association_file_columns(TRUE)` to read and parse the file. If the specifications contain columns with a type `"date"`, the function will parse the generic date with the format in the `dates_format` argument.

## See Also

`transform_columns`

`date_formats`

Other Import functions: `import_Vispa2_stats()`, `import_parallel_Vispa2Matrices()`, `import_single_Vispa2Matri`

## Examples

```
fs_path <- generate_default_folder_structure(type = "correct")
af <- import_association_file(fs_path$af,
    root = fs_path$root,
    report_path = NULL
)
head(af)
```

---

`import_ISA_settings` *Import a dynamic vars settings profile.*

---

## Description

The function allows the import of an existing dynamic vars profile in json format. This is a quick and convenient way to set up the workflow, alternative to specifying lookup tables manually through the corresponding setter functions. For more details, refer to the dedicated vignette `vignette("workflow_start", package="ISAnalytics")`.

## Usage

```
import_ISA_settings(path)
```

## Arguments

| | |
|---|---|
| `path` | The path to the json file on disk |

## Value

`NULL`

## See Also

Other Utilities: as_sparse_matrix(), comparison_matrix(), enable_progress_bars(), export_ISA_settings(), generate_Vispa2_launch_AF(), generate_blank_association_file(), generate_default_folder_structure(), separate_quant_matrices(), transform_columns()

## Examples

```
tmp_folder <- tempdir()
export_ISA_settings(tmp_folder, "DEFAULT")
import_ISA_settings(fs::path(tmp_folder, "DEFAULT_ISAsettings.json"))
reset_dyn_vars_config()
```

---

# import_parallel_Vispa2Matrices

*Import integration matrices from paths in the association file.*

---

## Description

**[Stable]** The function offers a convenient way of importing multiple integration matrices in an automated or semi-automated way. For more details see the "How to use import functions" vignette: `vignette("workflow_start", package = "ISAnalytics")`

## Usage

```
import_parallel_Vispa2Matrices(
  association_file,
  quantification_type = c("seqCount", "fragmentEstimate"),
  matrix_type = c("annotated", "not_annotated"),
  workers = 2,
  multi_quant_matrix = TRUE,
  report_path = default_report_path(),
  patterns = NULL,
  matching_opt = matching_options(),
  mode = "AUTO",
  ...
)
```

## Arguments

| | |
|---|---|
| association_file | Data frame imported via import_association_file (with file system alignment) |
| quantification_type | A vector of requested quantification_types. Possible choices are quantification_types |
| matrix_type | A single string representing the type of matrices to be imported. Can only be one in "annotated" or "not_annotated". |

| | |
|---|---|
| workers | A single integer representing the number of parallel workers to use for the import |
| multi_quant_matrix | If set to TRUE will produce a multi-quantification matrix through comparison_matrix instead of a list. |
| report_path | The path where the report file should be saved. Can be a folder or NULL if no report should be produced. Defaults to {user_home}/ISAnalytics_reports. |
| patterns | A character vector of additional patterns to match on file names. Please note that patterns must be regular expressions. Can be NULL if no patterns need to be matched. |
| matching_opt | A single value between matching_options |
| mode | Only AUTO is supported. As of ISAnalytics 1.8.3, the value INTERACTIVE is officially deprecated. |
| ... | <dynamic-dots> Additional named arguments to pass to comparison_matrix and import_single_Vispa2_matrix |

## Value

Either a multi-quantification matrix or a list of integration matrices

## Required tags

The function will explicitly check for the presence of these tags:

- project_id
- vispa_concatenate

## See Also

Other Import functions: import_Vispa2_stats(), import_association_file(), import_single_Vispa2Matrix()

## Examples

```
fs_path <- generate_default_folder_structure(type = "correct")
af <- import_association_file(fs_path$af,
    root = fs_path$root,
    report_path = NULL
)
matrices <- import_parallel_Vispa2Matrices(af,
    c("seqCount", "fragmentEstimate"),
    mode = "AUTO", report_path = NULL
)
head(matrices)
```

## import_parallel_Vispa2Matrices_auto

*Import integration matrices from association file.*

### Description

**[Defunct]** This function was deprecated to avoid redundancy. Please refer to `import_parallel_Vispa2Matrices`.

### Usage

```
import_parallel_Vispa2Matrices_auto(
  association_file,
  quantification_type,
  matrix_type = "annotated",
  workers = 2,
  multi_quant_matrix = TRUE,
  patterns = NULL,
  matching_opt = matching_options(),
  export_report_path = NULL,
  ...
)
```

### Value

A data frame or a list

## import_parallel_Vispa2Matrices_interactive

*Import integration matrices from association file.*

### Description

**[Defunct]** This function was deprecated to avoid redundancy. Please refer to `import_parallel_Vispa2Matrices`.

### Usage

```
import_parallel_Vispa2Matrices_interactive(
  association_file,
  quantification_type,
  matrix_type = "annotated",
  workers = 2,
  multi_quant_matrix = TRUE,
  export_report_path = NULL,
  ...
)
```

## Value

A data frame or a list

---

`import_single_Vispa2Matrix` *Import a single integration matrix from file*

---

## Description

**[Stable]** This function allows to read and import an integration matrix (ideally produced by VISPA2) and converts it to a tidy format.

## Usage

```
import_single_Vispa2Matrix(
  path,
  separator = "\t",
  additional_cols = NULL,
  transformations = NULL,
  sample_names_to = pcr_id_column(),
  values_to = "Value",
  to_exclude = lifecycle::deprecated(),
  keep_excluded = lifecycle::deprecated()
)
```

## Arguments

| | |
|---|---|
| `path` | The path to the file on disk |
| `separator` | The column delimiter used, defaults to `\t` |
| `additional_cols` | Either `NULL`, a named character vector or a named list. See details. |
| `transformations` | Either `NULL` or a named list of purrr-style lambdas where names are column names the function should be applied to. |
| `sample_names_to` | Name of the output column holding the sample identifier. Defaults to `pcr_id_column()` |
| `values_to` | Name of the output column holding the quantification values. Defaults to `Value`. |
| `to_exclude` | **[Deprecated]** Deprecated. Use `additonal_cols` instead |
| `keep_excluded` | **[Deprecated]** Deprecated. Use `additonal_cols` instead |

## Details

### Additional columns:

Additional columns are annotation columns present in the integration matrix to import that are not

- part of the mandatory IS vars (see `mandatory_IS_vars()`)
- part of the annotation IS vars (see `annotation_IS_vars()`)
- the sample identifier column
- the quantification column

When specified they tell the function how to treat those columns in the import phase, by providing a named character vector, where names correspond to the additional column names and values are a choice of the following:

- `"char"` for character (strings)
- `"int"` for integers
- `"logi"` for logical values (TRUE / FALSE)
- `"numeric"` for numeric values
- `"factor"` for factors
- `"date"` for generic date format - note that functions that need to read and parse files will try to guess the format and parsing may fail
- One of the accepted date/datetime formats by `lubridate`, you can use `ISAnalytics::date_formats()` to view the accepted formats
- `"_"` to drop the column

For more details see the "How to use import functions" vignette: `vignette("workflow_start", package = "ISAnalytics")`

### Transformations:

Lambdas provided in input in the `transformations` argument, must be transformations, aka functions that take in input a vector and return a vector of the same length as the input.

If the transformation list contains column names that are not present in the data frame, they are simply ignored.

## Value

A data frame object in tidy format

## Required tags

The function will explicitly check for the presence of these tags:

- All columns declared in `mandatory_IS_vars()`

## See Also

`transform_columns`

Other Import functions: `import_Vispa2_stats()`, `import_association_file()`, `import_parallel_Vispa2Matrices()`

## Examples

```
fs_path <- generate_default_folder_structure(type = "correct")
matrix_path <- fs::path(
    fs_path$root, "PJ01", "quantification",
    "POOL01-1", "PJ01_POOL01-1_seqCount_matrix.no0.annotated.tsv.gz"
)
matrix <- import_single_Vispa2Matrix(matrix_path)
head(matrix)
```

---

`import_Vispa2_stats` *Import Vispa2 stats given the aligned association file.*

---

## Description

**[Stable]** Imports all the Vispa2 stats files for each pool provided the association file has been aligned with the file system (see `import_association_file`).

## Usage

```
import_Vispa2_stats(
  association_file,
  file_prefixes = default_iss_file_prefixes(),
  join_with_af = TRUE,
  pool_col = "concatenatePoolIDSeqRun",
  report_path = default_report_path()
)
```

## Arguments

| | |
|---|---|
| `association_file` | The file system aligned association file (contains columns with absolute paths to the 'iss' folder) |
| `file_prefixes` | A character vector with known file prefixes to match on file names. NOTE: the elements represent regular expressions. For defaults see default_iss_file_prefixes. |
| `join_with_af` | Logical, if `TRUE` the imported stats files will be merged with the association file, if `FALSE` a single data frame holding only the stats will be returned. |
| `pool_col` | A single string. What is the name of the pool column used in the Vispa2 run? This will be used as a key to perform a join operation with the stats files `POOL` column. |
| `report_path` | The path where the report file should be saved. Can be a folder or `NULL` if no report should be produced. Defaults to `{user_home}/ISAnalytics_reports`. |

## Value

A data frame

### Required tags

The function will explicitly check for the presence of these tags:

- project_id
- tag_seq
- vispa_concatenate
- pcr_repl_id

### See Also

Other Import functions: `import_association_file()`, `import_parallel_Vispa2Matrices()`, `import_single_Vispa2Matrix()`

### Examples

```
fs_path <- generate_default_folder_structure(type = "correct")
af <- import_association_file(fs_path$af,
    root = fs_path$root,
    import_iss = FALSE,
    report_path = NULL
)
stats_files <- import_Vispa2_stats(af,
    join_with_af = FALSE,
    report_path = NULL
)
head(stats_files)
```

---

## inspect_tags *Retrieve description of a tag by name.*

---

### Description

Given one or multiple tags, prints the associated description and functions where the tag is explicitly used.

### Usage

```
inspect_tags(tags)
```

### Arguments

| | |
|---|---|
| tags | A character vector of tag names |

### Value

NULL

## See Also

Other dynamic vars: mandatory_IS_vars(), pcr_id_column(), reset_mandatory_IS_vars(), set_mandatory_IS_vars(), set_matrix_file_suffixes()

## Examples

```
inspect_tags(c("chromosome", "project_id", "x"))
```

---

`integration_alluvial_plot` *Alluvial plots for IS distribution in time.*

---

## Description

**[Stable]** Alluvial plots allow the visualization of integration sites distribution in different points in time in the same group. This functionality requires the suggested package ggalluvial.

## Usage

```
integration_alluvial_plot(
  x,
  group = c("SubjectID", "CellMarker", "Tissue"),
  plot_x = "TimePoint",
  plot_y = "fragmentEstimate_sum_PercAbundance",
  alluvia = mandatory_IS_vars(),
  alluvia_plot_y_threshold = 1,
  top_abundant_tbl = TRUE,
  empty_space_color = "grey90",
  ...
)
```

## Arguments

| | |
|---|---|
| `x` | A data frame. See details. |
| `group` | Character vector containing the column names that identify unique groups. |
| `plot_x` | Column name to plot on the x axis |
| `plot_y` | Column name to plot on the y axis |
| `alluvia` | Character vector of column names that uniquely identify alluvia |
| `alluvia_plot_y_threshold` | Numeric value. Everything below this threshold on y will be plotted in grey and aggregated. See details. |
| `top_abundant_tbl` | Logical. Produce the summary top abundant tables via top_abund_tableGrob? |

`empty_space_color`
: Color of the empty portion of the bars (IS below the threshold). Can be either a string of known colors, an hex code or `NA_character` to set the space transparent. All color specs accepted in ggplot2 are suitable here.

`...`
: Additional arguments to pass on to top_abund_tableGrob

## Details

### Input data frame:

The input data frame must contain all the columns specified in the arguments `group`, `plot_x`, `plot_y` and `alluvia`. The standard input for this function is the data frame obtained via the compute_abundance function.

### Plotting threshold on y:

The plotting threshold on the quantification on the y axis has the function to highlight only relevant information on the plot and reduce computation time. The default value is 1, that acts on the default column plotted on the y axis which contains a percentage value. This translates in natural language roughly as "highlight with colors only those integrations (alluvia) that at least in 1 point in time have an abundance value >= 1 %". The remaining integrations will be plotted as a unique layer in the column, colored as specified by the argument `empty_space_color`.

### Customizing the plot:

The returned plots are ggplot2 objects and can therefore further modified as any other ggplot2 object. For example, if the user decides to change the fill scale it is sufficient to do

```
plot +
  ggplot2::scale_fill_viridis_d(...) + # or any other discrete fill scale
  ggplot2::theme(...) # change theme options
```

NOTE: if you requested the computation of the top ten abundant tables and you want the colors to match you should re-compute them

### A note on strata ordering:

Strata in each column are ordered first by time of appearance and secondly in decreasing order of abundance (value of y). It means, for example, that if the plot has 2 or more columns, in the second column, on top, will appear first appear IS that appeared in the previous columns and then all other IS, ordered in decreasing order of abundance.

## Value

For each group a list with the associated plot and optionally the summary tableGrob

## See Also

Other Plotting functions: `CIS_volcano_plot()`, `HSC_population_plot()`, `circos_genomic_density()`, `fisher_scatterplot()`, `sharing_heatmap()`, `sharing_venn()`, `top_abund_tableGrob()`, `top_cis_overtime_heatmap`

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
abund <- compute_abundance(x = aggreg)
alluvial_plots <- integration_alluvial_plot(abund,
    alluvia_plot_y_threshold = 0.5
)
ex_plot <- alluvial_plots[[1]]$plot +
    ggplot2::labs(
        title = "IS distribution over time",
        subtitle = "Patient 1, MNC BM",
        y = "Abundance (%)",
        x = "Time point (days after GT)"
    )
print(ex_plot)
```

---

# integration_matrices *Example of imported multi-quantification integration matrices.*

---

## Description

The data was obtained manually by simulating real research data.

## Usage

```
data("integration_matrices")
```

## Format

Data frame with 1689 rows and 8 columns

**chr** The chromosome number (as character)

**integration_locus** Number of the base at which the viral insertion occurred

**strand** Strand of the integration

**GeneName** Symbol of the closest gene

**GeneStrand** Strand of the closest gene

**CompleteAmplificationID** Unique sample identifier

**seqCount** Value of the sequence count quantification

**fragmentEstimate** Value of the fragment estimate quantification

ISAnalytics *ISAnalytics: Analyze gene therapy vector insertion sites data identified from genomics next generation sequencing reads for clonal tracking studies*

## Description

**[Stable]** In gene therapy, stem cells are modified using viral vectors to deliver the therapeutic transgene and replace functional properties since the genetic modification is stable and inherited in all cell progeny. The retrieval and mapping of the sequences flanking the virus-host DNA junctions allows the identification of insertion sites (IS), essential for monitoring the evolution of genetically modified cells in vivo. A comprehensive toolkit for the analysis of IS is required to foster clonal trackign studies and supporting the assessment of safety and long term efficacy in vivo. This package is aimed at (1) supporting automation of IS workflow, (2) performing base and advance analysis for IS tracking (clonal abundance, clonal expansions and statistics for insertional mutagenesis, etc.), (3) providing basic biology insights of transduced stem cells in vivo.

## Useful resources

- VISPA2: A Scalable Pipeline for High-Throughput Identification and Annotation of Vector Integration Sites

## Vignettes

- `vignette("workflow_start", package = "ISAnalytics")`

## Author(s)

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## See Also

Useful links:

- https://calabrialab.github.io/ISAnalytics
- https://github.com//calabrialab/isanalytics
- https://calabrialab.github.io/ISAnalytics/
- Report bugs at https://github.com/calabrialab/ISAnalytics/issues

## ISAnalytics-deprecated

*Deprecated functions in package* **ISAnalytics**.

### Description

These functions are provided for compatibility with older versions of 'ISAnalytics' only, and will be defunct at the next release.

### Details

The following functions are deprecated and will be made defunct; use the replacement indicated below:

- import_parallel_Vispa2Matrices_auto (defunct): `import_parallel_Vispa2Matrices`
- import_parallel_Vispa2Matrices_interactive (defunct): `import_parallel_Vispa2Matrices`
- unzip_file_system: `generate_default_folder_structure`
- cumulative_count_union (defunct): `cumulative_is`
- threshold_filter

## iss_source

*Find the source of IS by evaluating sharing.*

### Description

**[Stable]** The function computes the sharing between a reference group of interest for each time point and a selection of groups of interest. In this way it is possible to observe the percentage of shared integration sites between reference and each group and identify in which time point a certain IS was observed for the first time.

### Usage

```
iss_source(
  reference,
  selection,
  ref_group_key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  selection_group_key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  timepoint_column = "TimePoint",
  by_subject = TRUE,
  subject_column = "SubjectID"
)
```

## Arguments

| | |
|---|---|
| reference | A data frame containing one or more groups of reference. Groups are identified by ref_group_key |
| selection | A data frame containing one or more groups of interest to compare. Groups are identified by selection_group_key |
| ref_group_key | Character vector of column names that identify a unique group in the reference data frame |
| selection_group_key | Character vector of column names that identify a unique group in the selection data frame |
| timepoint_column | Name of the column holding time point info? |
| by_subject | Should calculations be performed for each subject separately? |
| subject_column | Name of the column holding subjects information. Relevant only if by_subject = TRUE |

## Value

A list of data frames or a data frame

## See Also

Other Analysis functions: CIS_grubbs(), HSC_population_size_estimate(), compute_abundance(), cumulative_is(), gene_frequency_fisher(), is_sharing(), sample_statistics(), top_integrations(), top_targeted_genes()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
df1 <- aggreg |>
    dplyr::filter(.data$Tissue == "BM")
df2 <- aggreg |>
    dplyr::filter(.data$Tissue == "PB")
source <- iss_source(df1, df2)
source
ggplot2::ggplot(source$PT001, ggplot2::aes(
    x = as.factor(g2_TimePoint),
    y = sharing_perc, fill = g1
)) +
    ggplot2::geom_col() +
    ggplot2::labs(
        x = "Time point", y = "Shared IS % with MNC BM",
        title = "Source of is MNC BM vs MNC PB"
    )
```

`is_sharing` *Sharing of integration sites between given groups.*

## Description

**[Stable]** Computes the amount of integration sites shared between the groups identified in the input data.

## Usage

```
is_sharing(
  ...,
  group_key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  group_keys = NULL,
  n_comp = 2,
  is_count = TRUE,
  relative_is_sharing = TRUE,
  minimal = TRUE,
  include_self_comp = FALSE,
  keep_genomic_coord = FALSE,
  table_for_venn = FALSE
)
```

## Arguments

| | |
|---|---|
| `...` | One or more integration matrices |
| `group_key` | Character vector of column names which identify a single group. An associated group id will be derived by concatenating the values of these fields, separated by "_" |
| `group_keys` | A list of keys for asymmetric grouping. If not NULL the argument `group_key` is ignored |
| `n_comp` | Number of comparisons to compute. This argument is relevant only if provided a single data frame and a single key. |
| `is_count` | Logical, if `TRUE` returns also the count of IS for each group and the count for the union set |
| `relative_is_sharing` | Logical, if `TRUE` also returns the relative sharing. |
| `minimal` | Compute only combinations instead of all possible permutations? If `TRUE` saves time and excludes redundant comparisons. |
| `include_self_comp` | Include comparisons with the same group? |
| `keep_genomic_coord` | If `TRUE` keeps the genomic coordinates of the shared integration sites in a dedicated column (as a nested table) |
| `table_for_venn` | Add column with truth tables for venn plots? |

## Details

An integration site is always identified by the combination of fields in `mandatory_IS_vars()`, thus these columns must be present in the input(s).

The function accepts multiple inputs for different scenarios, please refer to the vignette `vignette("workflow_start", package = "ISAnalytics")` for a more in-depth explanation.

### Output:

The function outputs a single data frame containing all requested comparisons and optionally individual group counts, genomic coordinates of the shared integration sites and truth tables for plotting venn diagrams.

### Plotting sharing:

The sharing data obtained can be easily plotted in a heatmap via the function `sharing_heatmap` or via the function `sharing_venn`

## Value

A data frame

## Required tags

The function will explicitly check for the presence of these tags:

- All columns declared in `mandatory_IS_vars()`

## See Also

Other Analysis functions: `CIS_grubbs()`, `HSC_population_size_estimate()`, `compute_abundance()`, `cumulative_is()`, `gene_frequency_fisher()`, `iss_source()`, `sample_statistics()`, `top_integrations()`, `top_targeted_genes()`

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
sharing <- is_sharing(aggreg)
sharing
```

## known_clinical_oncogenes *Known clinical oncogenes (for mouse and human).*

### Description

Known clinical oncogenes (for mouse and human).

### Usage

```
known_clinical_oncogenes()
```

### Value

A data frame

### See Also

Other Plotting function helpers: `clinical_relevant_suspicious_genes()`

### Examples

```
known_clinical_oncogenes()
```

## mandatory_IS_vars *Current dynamic vars specifications getters.*

### Description

Fetches the look-up tables for different categories of dynamic vars. For more details, refer to the dedicated vignette `vignette("workflow_start", package="ISAnalytics")`.

- `mandatory_IS_vars` returns the look-up table of variables that are used to uniquely identify integration events
- `annotation_IS_vars()` returns the look-up table of variables that contain genomic annotations
- `association_file_columns()` returns the look-up table of variables that contains information on how metadata is structured
- `iss_stats_specs()` returns the look-up table of variables that contains information on the format of pool statistics files produced automatically by VISPA2
- `matrix_file_suffixes()` returns the look-up table of variables that contains all default file names for each quantification type and it is used by automated import functions

## Usage

```
mandatory_IS_vars(include_types = FALSE)

annotation_IS_vars(include_types = FALSE)

association_file_columns(include_types = FALSE)

iss_stats_specs(include_types = FALSE)

matrix_file_suffixes()
```

## Arguments

| | |
|---|---|
| include_types | If set to TRUE returns both the names and the types associated, otherwise returns only a character vector of names |

## Value

A character vector or a data frame

## See Also

Other dynamic vars: inspect_tags(), pcr_id_column(), reset_mandatory_IS_vars(), set_mandatory_IS_vars(), set_matrix_file_suffixes()

## Examples

```
# Names only
mandatory_IS_vars()

# Names and types
mandatory_IS_vars(TRUE)

# Names only
annotation_IS_vars()

# Names and types
annotation_IS_vars(TRUE)

# Names only
association_file_columns()

# Names and types
association_file_columns(TRUE)

# Names only
iss_stats_specs()

# Names and types
iss_stats_specs(TRUE)
```

```
# Names only
matrix_file_suffixes()
```

---

`matching_options` *Possible choices for the* `matching_opt` *parameter.*

---

## Description

These are all the possible values for the `matching_opt` parameter in `import_parallel_vispa2Matrices_auto`.

## Usage

```
matching_options()
```

## Details

The values "ANY", "ALL" and "OPTIONAL", represent how the patterns should be matched, more specifically

- ANY = look only for files that match AT LEAST one of the patterns specified
- ALL = look only for files that match ALL of the patterns specified
- OPTIONAL = look preferentially for files that match, in order, all patterns or any pattern and if no match is found return what is found (keep in mind that duplicates are discarded in automatic mode)

## Value

A vector of characters for matching_opt

## See Also

`import_parallel_Vispa2Matrices_auto`

Other Import functions helpers: `annotation_issues()`, `date_formats()`, `default_af_transform()`, `default_iss_file_prefixes()`, `quantification_types()`

## Examples

```
opts <- matching_options()
```

## NGSdataExplorer *Launch the shiny application NGSdataExplorer.*

### Description

Launch the shiny application NGSdataExplorer.

### Usage

```
NGSdataExplorer()
```

### Value

Nothing

### Examples

```
## Not run:
NGSdataExplorer()

## End(Not run)
```

## outliers_by_pool_fragments *Identify and flag outliers based on pool fragments.*

### Description

**[Stable]** Identify and flag outliers based on expected number of raw reads per pool.

### Usage

```
outliers_by_pool_fragments(
  metadata,
  key = "BARCODE_MUX",
  outlier_p_value_threshold = 0.01,
  normality_test = FALSE,
  normality_p_value_threshold = 0.05,
  transform_log2 = TRUE,
  per_pool_test = TRUE,
  pool_col = "PoolID",
  min_samples_per_pool = 5,
  flag_logic = "AND",
  keep_calc_cols = TRUE,
  report_path = default_report_path()
)
```

## Arguments

| | |
|---|---|
| metadata | The metadata data frame |
| key | A character vector of numeric column names |
| outlier_p_value_threshold | The p value threshold for a read to be considered an outlier |
| normality_test | Perform normality test? Normality is assessed for each column in the key using Shapiro-Wilk test and if the values do not follow a normal distribution, other calculations are skipped |
| normality_p_value_threshold | Normality threshold |
| transform_log2 | Perform a log2 trasformation on values prior the actual calculations? |
| per_pool_test | Perform the test for each pool? |
| pool_col | A character vector of the names of the columns that uniquely identify a pool |
| min_samples_per_pool | The minimum number of samples that a pool needs to contain in order to be processed - relevant only if per_pool_test = TRUE |
| flag_logic | A character vector of logic operators to obtain a global flag formula - only relevant if the key is longer than one. All operators must be chosen between: AND, OR, XOR, NAND, NOR, XNOR |
| keep_calc_cols | Keep the calculation columns in the output data frame? |
| report_path | The path where the report file should be saved. Can be a folder, a file or NULL if no report should be produced. Defaults to {user_home}/ISAnalytics_reports. |

## Details

**Modular structure:**

The outlier filtering functions are structured in a modular fashion. There are 2 kind of functions:

- Outlier tests - Functions that perform some kind of calculation based on inputs and flags metadata
- Outlier filter - A function that takes one or more outlier tests, combines all the flags with a given logic and filters out rows that are flagged as outliers

This function is an outlier test, and calculates for each column in the key

- The zscore of the values
- The tstudent of the values
- The the associated p-value (tdist)

Optionally the test can be performed for each pool and a normality test can be run prior the actual calculations. Samples are flagged if this condition is respected:

- tdist < outlier_p_value_threshold & zscore < 0

If the key contains more than one column an additional flag logic can be specified for combining the results. Example: let's suppose the key contains the names of two columns, X and Y key = c("X", "Y") if we specify the the argument flag_logic = "AND" then the reads will be flagged based on this global condition: (tdist_X < outlier_p_value_threshold & zscore_X < 0) AND (tdist_Y < outlier_p_value_threshold & zscore_Y < 0)

The user can specify one or more logical operators that will be applied in sequence.

## Value

A data frame of metadata with the column `to_remove`

## See Also

Other Data cleaning and pre-processing: `aggregate_metadata()`, `aggregate_values_by_key()`, `compute_near_integrations()`, `default_meta_agg()`, `outlier_filter()`, `purity_filter()`, `realign_after_collisions()`, `remove_collisions()`, `threshold_filter()`

## Examples

```
data("association_file", package = "ISAnalytics")
flagged <- outliers_by_pool_fragments(association_file,
    report_path = NULL
)
head(flagged)
```

---

`outlier_filter` *Filter out outliers in metadata, identified by the chosen outlier test.*

---

## Description

**[Experimental]** Filter out outliers in metadata by using appropriate outlier tests.

## Usage

```
outlier_filter(
  metadata,
  pcr_id_col = pcr_id_column(),
  outlier_test = c(outliers_by_pool_fragments),
  outlier_test_outputs = NULL,
  combination_logic = c("AND"),
  negate = FALSE,
  report_path = default_report_path(),
  ...
)
```

## Arguments

| | |
|---|---|
| `metadata` | The metadata data frame |
| `pcr_id_col` | The name of the pcr identifier column |
| `outlier_test` | One or more outlier tests. Must be functions, either from `available_outlier_tests()` or custom functions that produce an appropriate output format (see details). |
| `outlier_test_outputs` | `NULL`, a data frame or a list of data frames. See details. |

`combination_logic` One or more logical operators ("AND", "OR", "XOR", "NAND", "NOR", "XNOR"). See datails.

`negate` If `TRUE` will return only the metadata that was flagged to be removed. If `FALSE` will return only the metadata that wasn't flagged to be removed.

`report_path` The path where the report file should be saved. Can be a folder or `NULL` if no report should be produced. Defaults to `{user_home}/ISAnalytics_reports`.

`...` Additional named arguments passed to `outliers_test`

## Details

### Modular structure:

The outlier filtering functions are structured in a modular fashion. There are 2 kind of functions:

- Outlier tests - Functions that perform some kind of calculation based on inputs and flags metadata
- Outlier filter - A function that takes one or more outlier tests, combines all the flags with a given logic and filters out rows that are flagged as outliers

This function acts as the filter. It can either take one or more outlier tests as functions and call them through the argument `outlier_test`, or it can take directly outputs produced by individual tests in the argument `outlier_test_outputs` - if both are provided the second one has priority. The second method offers a bit more freedom, since single tests can be run independently and intermediate results saved and examined more in detail. If more than one test is to be performed, the argument `combination_logic` tells the function how to combine the flags: you can specify 1 logical operator or more than 1, provided it is compatible with the number of tests.

### Writing custom outlier tests:

You have the freedom to provide your own functions as outlier tests. For this purpose, functions provided must respect this guidelines:

- Must take as input the whole metadata df
- Must return a df containing AT LEAST the `pcr_id_col` and a logical column `"to_remove"` that contains the flag
- The `pcr_id_col` must contain all the values originally present in the metadata df

## Value

A data frame of metadata which has less or the same amount of rows

## See Also

Other Data cleaning and pre-processing: `aggregate_metadata()`, `aggregate_values_by_key()`, `compute_near_integrations()`, `default_meta_agg()`, `outliers_by_pool_fragments()`, `purity_filter()`, `realign_after_collisions()`, `remove_collisions()`, `threshold_filter()`

## Examples

```
data("association_file", package = "ISAnalytics")
filtered_af <- outlier_filter(association_file,
    key = "BARCODE_MUX",
    report_path = NULL
)
head(filtered_af)
```

---

`pcr_id_column` *Easily retrieve the name of the pcr id column.*

---

## Description

The function is a shortcut to retrieve the currently set pcr id column name from the association file column tags look-up table. This column is needed every time a joining operation with metadata needs to be performed

## Usage

```
pcr_id_column()
```

## Value

The name of the column

## See Also

Other dynamic vars: `inspect_tags()`, `mandatory_IS_vars()`, `reset_mandatory_IS_vars()`, `set_mandatory_IS_vars()`, `set_matrix_file_suffixes()`

## Examples

```
pcr_id_column()
```

---

`proto_oncogenes` *Data frames for proto-oncogenes (human and mouse) amd tumor-suppressor genes from UniProt.*

---

## Description

The file is simply a result of a research with the keywords "proto-oncogenes" and "tumor suppressor" for the target genomes on UniProt database.

### Usage

```
data("proto_oncogenes")

data("tumor_suppressors")
```

### Format

An object of class `tbl_df` (inherits from `tbl`, `data.frame`) with 569 rows and 13 columns.

An object of class `tbl_df` (inherits from `tbl`, `data.frame`) with 523 rows and 13 columns.

### Functions

- `tumor_suppressors`: Data frame for tumor suppressor genes

---

## purity_filter *Filter integration sites based on purity.*

---

### Description

**[Stable]** Filter that targets possible contamination between cell lines based on a numeric quantification (likely abundance or sequence count).

### Usage

```
purity_filter(
  x,
  lineages = blood_lineages_default(),
  aggregation_key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  group_key = c("CellMarker", "Tissue"),
  selected_groups = NULL,
  join_on = "CellMarker",
  min_value = 3,
  impurity_threshold = 10,
  by_timepoint = TRUE,
  timepoint_column = "TimePoint",
  value_column = "seqCount_sum"
)
```

### Arguments

| | |
|---|---|
| `x` | An aggregated integration matrix, obtained via `aggregate_values_by_key()` |
| `lineages` | A data frame containing cell lineages information |
| `aggregation_key` | The key used for aggregating `x` |
| `group_key` | A character vector of column names for re-aggregation. Column names must be either in `x` or in `lineages`. See details. |

selected_groups
: Either NULL, a character vector or a data frame for group selection. See details.

join_on
: Common columns to perform a join operation on

min_value
: A minimum value to filter the input matrix. Integrations with a value strictly lower than min_value are excluded (dropped) from the output.

impurity_threshold
: The ratio threshold for impurity in groups

by_timepoint
: Should filtering be applied on each time point? If FALSE, all time points are merged together

timepoint_column
: Column in x containing the time point

value_column
: Column in x containing the numeric quantification of interest

## Details

### Setting input arguments:

The input matrix can be re-aggregated with the provided group_key argument. This key contains the names of the columns to group on (besides the columns holding genomic coordinates of the integration sites) and must be contained in at least one of x or lineages data frames. If the key is not found only in x, then a join operation with the lineages data frame is performed on the common column(s) join_on.

### Group selection:

It is possible for the user to specify on which groups the logic of the filter should be applied to. For example: if we have group_key = c("HematoLineage") and we set selected_groups = c("CD34", "Myeloid","Lymphoid") it means that a single integration will be evaluated for the filter only for groups that have the values of "CD34", "Myeloid" and "Lymphoid" in the "HematoLineage" column. If the same integration is present in other groups it is kept as it is. selected_groups can be set to NULL if we want the logic to apply to every group present in the data frame, it can be set as a simple character vector as the example above if the group key has length 1 (and there is no need to filter on time point). If the group key is longer than 1 then the filter is applied only on the first element of the key.

If a more refined selection on groups is needed, a data frame can be provided instead:

```
group_key = c("CellMarker", "Tissue")
selected_groups = tibble::tribble(
~ CellMarker, ~ Tissue,
"CD34", "BM",
"CD14", "BM",
"CD14", "PB"
)
```

Columns in the data frame should be the same as group key (plus, eventually, the time point column). In this example only those groups identified by the rows in the provided data frame are processed.

## Value

A data frame

## See Also

Other Data cleaning and pre-processing: aggregate_metadata(), aggregate_values_by_key(), compute_near_integrations(), default_meta_agg(), outlier_filter(), outliers_by_pool_fragments(), realign_after_collisions(), remove_collisions(), threshold_filter()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
filtered_by_purity <- purity_filter(
    x = aggreg,
    value_column = "seqCount_sum"
)
head(filtered_by_purity)
```

---

# quantification_types *Possible choices for the* quantification_type *parameter.*

---

## Description

These are all the possible values for the quantification_type parameter in import_parallel_vispa2Matrices_interac
and import_parallel_vispa2Matrices_auto.

## Usage

```
quantification_types()
```

## Details

The possible values are:

- fragmentEstimate
- seqCount
- barcodeCount
- cellCount
- ShsCount

## Value

A vector of characters for quantification types

## See Also

import_parallel_Vispa2Matrices_interactive, import_parallel_Vispa2Matrices_auto

Other Import functions helpers: annotation_issues(), date_formats(), default_af_transform(), default_iss_file_prefixes(), matching_options()

## Examples

```
quant_types <- quantification_types()
```

---

# realign_after_collisions

*Re-aligns matrices of other quantification types based on the processed sequence count matrix.*

---

## Description

**[Stable]** This function should be used to keep data consistent among the same analysis: if for some reason you removed the collisions by passing only the sequence count matrix to `remove_collisions()`, you should call this function afterwards, providing a list of other quantification matrices. NOTE: if you provided a list of several quantification types to `remove_collisions()` before, there is no need to call this function.

## Usage

```
realign_after_collisions(
  sc_matrix,
  other_matrices,
  sample_column = pcr_id_column()
)
```

## Arguments

| | |
|---|---|
| `sc_matrix` | The sequence count matrix already processed for collisions via `remove_collisions()` |
| `other_matrices` | A named list of matrices to re-align. Names in the list must be quantification types (`quantification_types()`) except "seqCount". |
| `sample_column` | The name of the column containing the sample identifier |

## Details

For more details on how to use collision removal functionality: `vignette("workflow_start", package = "ISAnalytics")`

## Value

A named list with re-aligned matrices

## See Also

remove_collisions

Other Data cleaning and pre-processing: aggregate_metadata(), aggregate_values_by_key(), compute_near_integrations(), default_meta_agg(), outlier_filter(), outliers_by_pool_fragments(), purity_filter(), remove_collisions(), threshold_filter()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
separated <- separate_quant_matrices(
    integration_matrices
)
no_coll <- remove_collisions(
    x = separated$seqCount,
    association_file = association_file,
    quant_cols = c(seqCount = "Value"),
    report_path = NULL
)
realigned <- realign_after_collisions(
    sc_matrix = no_coll,
    other_matrices = list(fragmentEstimate = separated$fragmentEstimate)
)
realigned
```

---

# reduced_AF_columns *Names of the columns of the association file to consider for Vispa2 launch.*

---

## Description

Selection of column names from the association file to be considered for Vispa2 launch. NOTE: the TagID column appears only once but needs to be repeated twice for generating the launch file. Use the appropriate function to generate the file automatically.

## Usage

```
reduced_AF_columns()
```

## Value

A character vector

## Examples

```
reduced_AF_columns()
```

refGenes_hg19 *Gene annotation files for hg19, mm9 and mm10.*

## Description

This file was obtained following this steps:

1. Download from http://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/ the refGene.sql, knownGene.sql, knownToRefSeq.sql, kgXref.sql tables
2. Import everything it in mysql
3. Generate views for annotation:

```
SELECT kg.`chrom`, min(kg.cdsStart) as CDS_minStart,
max(kg.`cdsEnd`) as CDS_maxEnd, k2a.geneSymbol,
kg.`strand` as GeneStrand, min(kg.txStart) as TSS_minStart,
max(kg.txEnd) as TSS_maxStart,
kg.proteinID as ProteinID, k2a.protAcc as ProteinAcc, k2a.spDisplayID
FROM `knownGene` AS kg JOIN kgXref AS k2a
ON BINARY kg.name = k2a.kgID COLLATE latin1_bin
-- latin1_swedish_ci
-- WHERE k2a.spDisplayID IS NOT NULL and (k2a.`geneSymbol` LIKE 'Tcra%' or
k2a.`geneSymbol` LIKE 'TCRA%')
WHERE (k2a.spDisplayID IS NOT NULL or k2a.spDisplayID NOT LIKE '')
and k2a.`geneSymbol` LIKE 'Tcra%'
group by kg.`chrom`, k2a.geneSymbol
ORDER BY kg.chrom ASC , kg.txStart ASC
```

## Usage

```
data("refGenes_hg19")

data("refGenes_mm9")
```

## Format

An object of class `tbl_df` (inherits from `tbl`, `data.frame`) with 27275 rows and 12 columns.

An object of class `tbl_df` (inherits from `tbl`, `data.frame`) with 24487 rows and 12 columns.

## Functions

- `refGenes_mm9`: Data frame for murine mm9 genome

## refGene_table_cols *Required columns for refGene file.*

### Description

Required columns for refGene file.

### Usage

```
refGene_table_cols()
```

### Value

Character vector of column names

### Examples

```
refGene_table_cols()
```

## remove_collisions *Identifies and removes collisions.*

### Description

**[Stable]** A collision is an integration (aka a unique combination of the provided `mandatory_IS_vars()`) which is observed in more than one independent sample. The function tries to decide to which independent sample should an integration event be assigned to, and if no decision can be taken, the integration is completely removed from the data frame. For more details refer to the vignette "Collision removal functionality": `vignette("workflow_start", package = "ISAnalytics")`

### Usage

```
remove_collisions(
  x,
  association_file,
  independent_sample_id = c("ProjectID", "SubjectID"),
  date_col = "SequencingDate",
  reads_ratio = 10,
  quant_cols = c(seqCount = "seqCount", fragmentEstimate = "fragmentEstimate"),
  report_path = default_report_path(),
  max_workers = NULL
)
```

## Arguments

| | |
|---|---|
| x | Either a multi-quantification matrix (recommended) or a named list of matrices (names must be quantification types) |
| association_file | The association file imported via import_association_file() |
| independent_sample_id | A character vector of column names that identify independent samples |
| date_col | The date column that should be considered. |
| reads_ratio | A single numeric value that represents the ratio that has to be considered when deciding between seqCount value. |
| quant_cols | A named character vector where names are quantification types and values are the names of the corresponding columns. The quantification seqCount MUST be included in the vector. |
| report_path | The path where the report file should be saved. Can be a folder or NULL if no report should be produced. Defaults to {user_home}/ISAnalytics_reports. |
| max_workers | Maximum number of parallel workers to distribute the workload. If NULL (default) produces the maximum amount of workers allowed, a numeric value is requested otherwise. WARNING: a higher number of workers speeds up computation at the cost of memory consumption! Tune this parameter accordingly. |

## Value

Either a multi-quantification matrix or a list of data frames

## Required tags

The function will explicitly check for the presence of these tags:

- project_id
- pool_id
- pcr_replicate

## See Also

Other Data cleaning and pre-processing: aggregate_metadata(), aggregate_values_by_key(), compute_near_integrations(), default_meta_agg(), outlier_filter(), outliers_by_pool_fragments(), purity_filter(), realign_after_collisions(), threshold_filter()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
no_coll <- remove_collisions(
    x = integration_matrices,
    association_file = association_file,
    report_path = NULL
)
head(no_coll)
```

`reset_mandatory_IS_vars` *Resets dynamic vars to the default values.*

## Description

Reverts all changes to dynamic vars to the default values. For more details, refer to the dedicated vignette `vignette("workflow_start", package="ISAnalytics")`.

- `reset_mandatory_IS_vars()` re-sets the look-up table for mandatory IS vars.
- `reset_annotation_IS_vars()` re-sets the look-up table for genomic annotation IS vars.
- `reset_af_columns_def()` re-sets the look-up table for association file columns vars
- `reset_iss_stats_specs()` re-sets the look-up table for VISPA2 pool statistics vars
- `reset_matrix_file_suffixes()` re-sets the matrix file suffixes look-up table
- `reset_dyn_vars_config()` re-sets all look-up tables

## Usage

```
reset_mandatory_IS_vars()

reset_annotation_IS_vars()

reset_af_columns_def()

reset_iss_stats_specs()

reset_matrix_file_suffixes()

reset_dyn_vars_config()
```

## Value

`NULL`

## See Also

Other dynamic vars: `inspect_tags()`, `mandatory_IS_vars()`, `pcr_id_column()`, `set_mandatory_IS_vars()`, `set_matrix_file_suffixes()`

## Examples

```
reset_mandatory_IS_vars()

reset_annotation_IS_vars()

reset_af_columns_def()

reset_iss_stats_specs()

reset_matrix_file_suffixes()

reset_dyn_vars_config()
```

---

`sample_statistics` *Computes user specified functions on numerical columns and updates the metadata data frame accordingly.*

---

## Description

**[Stable]** The function operates on a data frame by grouping the content by the sample key and computing every function specified on every column in the `value_columns` parameter. After that the metadata data frame is updated by including the computed results as columns for the corresponding key. For this reason it's required that both `x` and `metadata` have the same sample key, and it's particularly important if the user is working with previously aggregated data. For example:

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
 x = integration_matrices,
 association_file = association_file,
 value_cols = c("seqCount", "fragmentEstimate")
)
aggreg_meta <- aggregate_metadata(association_file = association_file)

sample_stats <- sample_statistics(x = aggreg,
metadata = aggreg_meta,
value_columns = c("seqCount", "fragmentEstimate"),
sample_key = c("SubjectID", "CellMarker","Tissue", "TimePoint"))
```

## Usage

```
sample_statistics(
  x,
  metadata,
  sample_key = "CompleteAmplificationID",
  value_columns = "Value",
```

```
  functions = default_stats(),
  add_integrations_count = TRUE
)
```

## Arguments

| | |
|---|---|
| x | A data frame |
| metadata | The metadata data frame |
| sample_key | Character vector representing the key for identifying a sample |
| value_columns | The name of the columns to be computed, must be numeric or integer |
| functions | A named list of function or purrr-style lambdas |
| add_integrations_count | Add the count of distinct integration sites for each group? Can be computed only if x contains the mandatory columns mandatory_IS_vars() |

## Value

A list with modified x and metadata data frames

## Required tags

The function will explicitly check for the presence of these tags:

- All columns declared in mandatory_IS_vars()

These are checked only if add_integrations_count = TRUE.

## See Also

Other Analysis functions: CIS_grubbs(), HSC_population_size_estimate(), compute_abundance(), cumulative_is(), gene_frequency_fisher(), is_sharing(), iss_source(), top_integrations(), top_targeted_genes()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
stats <- sample_statistics(
    x = integration_matrices,
    metadata = association_file,
    value_columns = c("seqCount", "fragmentEstimate")
)
stats
```

separate_quant_matrices *Separate a multiple-quantification matrix into single quantification matrices.*

## Description

**[Stable]** The function separates a single multi-quantification integration matrix, obtained via comparison_matrix, into single quantification matrices as a named list of tibbles.

## Usage

```
separate_quant_matrices(
  x,
  fragmentEstimate = "fragmentEstimate",
  seqCount = "seqCount",
  barcodeCount = "barcodeCount",
  cellCount = "cellCount",
  ShsCount = "ShsCount",
  key = c(mandatory_IS_vars(), annotation_IS_vars(), "CompleteAmplificationID")
)
```

## Arguments

| | |
|---|---|
| x | Single integration matrix with multiple quantification value columns, obtained via comparison_matrix. |
| fragmentEstimate | Name of the fragment estimate values column in input |
| seqCount | Name of the sequence count values column in input |
| barcodeCount | Name of the barcode count values column in input |
| cellCount | Name of the cell count values column in input |
| ShsCount | Name of the shs count values column in input |
| key | Key columns to perform the joining operation |

## Value

A named list of data frames, where names are quantification types

## See Also

quantification_types

Other Utilities: as_sparse_matrix(), comparison_matrix(), enable_progress_bars(), export_ISA_settings(), generate_Vispa2_launch_AF(), generate_blank_association_file(), generate_default_folder_structure(), import_ISA_settings(), transform_columns()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
separated <- separate_quant_matrices(
    integration_matrices
)
```

---

`set_mandatory_IS_vars` *Define custom dynamic vars.*

---

## Description

This set of function allows users to specify custom look-up tables for dynamic variables. For more details, refer to the dedicated vignette `vignette("workflow_start", package="ISAnalytics")`.

- `set_mandatory_IS_vars()` sets the look-up table for mandatory IS vars.
- `set_annotation_IS_vars()` sets the look-up table for genomic annotation IS vars.
- `set_af_columns_def()` sets the look-up table for association file columns vars
- `set_iss_stats_specs()` sets the look-up table for VISPA2 pool statistics vars

## Usage

```
set_mandatory_IS_vars(specs)

set_annotation_IS_vars(specs)

set_af_columns_def(specs)

set_iss_stats_specs(specs)
```

## Arguments

| | |
|---|---|
| `specs` | Either a named vector or a data frame with specific format. See details. |

## Details

The user can supply specifications in the form of a named vector or a data frame.

**Named vector:**

When using a named vector, names should be the names of the columns, values should be the type associated with each column in the form of a string. The vector gets automatically converted into a data frame with the right format (default values for the columns `transform` and `flag` are `NULL` and `required` respectively). Use of this method is however discouraged: data frame inputs are preferred since they offer more control.

**Look-up table structure:**

The look-up table for dynamic vars should always follow this structure:

| names | types | transform | flag | tag |
|---|---|---|---|---|
| <name of the column> | <type> | <a lambda or NULL> | <flag> | <tag> |

where

- `names` contains the name of the column as a character
- `types` contains the type of the column. Type should be expressed as a string and should be in one of the allowed types
- `char` for character (strings)
- `int` for integers
- `logi` for logical values (TRUE / FALSE)
- `numeric` for numeric values
- `factor` for factors
- `date` for generic date format - note that functions that need to read and parse files will try to guess the format and parsing may fail
- One of the accepted date/datetime formats by `lubridate`, you can use `ISAnalytics::date_formats()` to view the accepted formats
- `transform`: a purrr-style lambda that is applied immediately after importing. This is useful to operate simple transformations like removing unwanted characters or rounding to a certain precision. Please note that these lambdas need to be functions that accept a vector as input and only operate a transformation, aka they output a vector of the same length as the input. For more complicated applications that may require the value of other columns, appropriate functions should be manually applied post-import.
- `flag`: as of now, it should be set either to `required` or `optional` - some functions internally check for only required tags presence and if those are missing from inputs they fail, signaling failure to the user
- `tag`: a specific tag expressed as a string

**Column types::**

Type should be expressed as a string and should be in one of the allowed types

- `char` for character (strings)
- `int` for integers
- `logi` for logical values (TRUE / FALSE)
- `numeric` for numeric values
- `factor` for factors
- `date` for generic date format - note that functions that need to read and parse files will try to guess the format and parsing may fail
- One of the accepted date/datetime formats by `lubridate`, you can use `ISAnalytics::date_formats()` to view the accepted formats

## Value

`NULL`

## See Also

Other dynamic vars: inspect_tags(), mandatory_IS_vars(), pcr_id_column(), reset_mandatory_IS_vars(), set_matrix_file_suffixes()

## Examples

```
tmp_mand_vars <- tibble::tribble(
    ~names, ~types, ~transform, ~flag, ~tag,
    "chrom", "char", ~ stringr::str_replace_all(.x, "chr", ""), "required",
    "chromosome",
    "position", "int", NULL, "required", "locus",
    "strand", "char", NULL, "required", "is_strand",
    "gap", "int", NULL, "required", NA_character_,
    "junction", "int", NULL, "required", NA_character_
)
set_mandatory_IS_vars(tmp_mand_vars)
print(mandatory_IS_vars(TRUE))
reset_mandatory_IS_vars()

tmp_annot_vars <- tibble::tribble(
    ~names, ~types, ~transform, ~flag, ~tag,
    "gene", "char", NULL, "required",
    "gene_symbol",
    "gene_strand", "char", NULL, "required", "gene_strand"
)
print(annotation_IS_vars(TRUE))
reset_annotation_IS_vars()

temp_af_cols <- tibble::tribble(
    ~names, ~types, ~transform, ~flag, ~tag,
    "project", "char", NULL, "required",
    "project_id",
    "pcr_id", "char", NULL, "required", "pcr_repl_id",
    "subject", "char", NULL, "required", "subject"
)
set_af_columns_def(temp_af_cols)
print(association_file_columns(TRUE))
reset_af_columns_def()

tmp_iss_vars <- tibble::tribble(
    ~names, ~types, ~transform, ~flag, ~tag,
    "pool", "char", NULL, "required",
    "vispa_concatenate",
    "tag", "char", NULL, "required", "tag_seq",
    "barcode", "int", NULL, "required", NA_character_
)
set_iss_stats_specs(tmp_iss_vars)
iss_stats_specs(TRUE)
reset_iss_stats_specs()
```

`set_matrix_file_suffixes` *Sets the look-up table for matrix file suffixes.*

## Description

The function automatically produces and sets a look-up table of matrix file suffixes based on user input.

## Usage

```
set_matrix_file_suffixes(
  quantification_suffix = list(seqCount = "seqCount", fragmentEstimate =
   "fragmentEstimate", barcodeCount = "barcodeCount", cellCount = "cellCount", ShsCount
    = "ShsCount"),
  annotation_suffix = list(annotated = ".no0.annotated", not_annotated = ""),
  file_ext = "tsv.gz",
 glue_file_spec = "{quantification_suffix}_matrix{annotation_suffix}.{file_ext}"
)
```

## Arguments

| | |
|---|---|
| `quantification_suffix` | A named list - names must be quantification types in `quantification_types()`, and values must be single strings, containing the associated suffix. Please note that ALL quantification types must be specified or the function will produce an error. |
| `annotation_suffix` | A named list - names must be `annotated` and `not_annotated`, values must be single strings, containing the associated suffix. Please note that both names must be present in the list or the function will produce an error. |
| `file_ext` | The file extension (e.g. `tsv`, `tsv.gz`) |
| `glue_file_spec` | A string specifying the pattern used to form the entire suffix, as per `glue::glue()` requirements. The string should contain the reference to `quantification_suffix`, `annotation_suffix` and `file_ext`. |

## Value

`NULL`

## See Also

Other dynamic vars: `inspect_tags()`, `mandatory_IS_vars()`, `pcr_id_column()`, `reset_mandatory_IS_vars()`, `set_mandatory_IS_vars()`

## Examples

```
set_matrix_file_suffixes(
    quantification_suffix = list(
        seqCount = "sc",
        fragmentEstimate = "fe",
        barcodeCount = "barcodeCount",
        cellCount = "cellCount",
        ShsCount = "ShsCount"
    ),
    annotation_suffix = list(annotated = "annot", not_annotated = "")
)
matrix_file_suffixes()
reset_matrix_file_suffixes()
```

---

# sharing_heatmap *Plot IS sharing heatmaps.*

---

## Description

**[Stable]** Displays the IS sharing calculated via is_sharing as heatmaps.

## Usage

```
sharing_heatmap(
  sharing_df,
  show_on_x = "g1",
  show_on_y = "g2",
  absolute_sharing_col = "shared",
  title_annot = NULL,
  plot_relative_sharing = TRUE,
  rel_sharing_col = c("on_g1", "on_union"),
  show_perc_symbol_rel = TRUE,
  interactive = FALSE
)
```

## Arguments

| | |
|---|---|
| sharing_df | The data frame containing the IS sharing data |
| show_on_x | Name of the column to plot on the x axis |
| show_on_y | Name of the column to plot on the y axis |
| absolute_sharing_col | Name of the column that contains the absolute values of IS sharing |
| title_annot | Additional text to display in the title |
| plot_relative_sharing | Logical. Compute heatmaps also for relative sharing? |

rel_sharing_col
: Names of the columns to consider as relative sharing. The function is going to plot one heatmap per column in this argument.

show_perc_symbol_rel
: Logical. Only relevant if plot_relative_sharing is set to TRUE, should the percentage symbol be displayed in relative heatmaps?

interactive
: Logical. Requires the package plotly is required for this functionality. Returns the heatmaps as interactive HTML widgets.

## Value

A list of plots or widgets

## See Also

is_sharing

Other Plotting functions: CIS_volcano_plot(), HSC_population_plot(), circos_genomic_density(), fisher_scatterplot(), integration_alluvial_plot(), sharing_venn(), top_abund_tableGrob(), top_cis_overtime_heatmap()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
sharing <- is_sharing(aggreg,
    minimal = FALSE,
    include_self_comp = TRUE
)
sharing_heatmaps <- sharing_heatmap(sharing_df = sharing)
sharing_heatmaps$absolute
sharing_heatmaps$on_g1
sharing_heatmaps$on_union
```

---

# sharing_venn *Produce tables to plot sharing venn or euler diagrams.*

---

## Description

**[Stable]** This function processes a sharing data frame obtained via is_sharing() with the option table_for_venn = TRUE to obtain a list of objects that can be plotted as venn or euler diagrams.

## Usage

```
sharing_venn(sharing_df, row_range = NULL, euler = TRUE)
```

### Arguments

| | |
|---|---|
| sharing_df | The sharing data frame |
| row_range | Either NULL or a numeric vector of row indexes (e.g. c(1, 4, 5) will produce tables only for rows 1, 4 and 5) |
| euler | If TRUE will produce tables for euler diagrams, otherwise will produce tables for venn diagrams |

### Details

The functions requires the package eulerr. Each row of the input data frame is representable as a venn/euler diagram. The function allows to specify a range of row indexes to obtain a list of plottable objects all at once, leave it to NULL to process all rows.

To actually plot the data it is sufficient to call the function `plot()` and specify optional customization arguments. See eulerr docs for more detail on this.

### Value

A list of data frames

### See Also

Other Plotting functions: `CIS_volcano_plot()`, `HSC_population_plot()`, `circos_genomic_density()`, `fisher_scatterplot()`, `integration_alluvial_plot()`, `sharing_heatmap()`, `top_abund_tableGrob()`, `top_cis_overtime_heatmap()`

### Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
sharing <- is_sharing(aggreg, n_comp = 3, table_for_venn = TRUE)
venn_tbls <- sharing_venn(sharing, row_range = 1:3, euler = FALSE)
venn_tbls
plot(venn_tbls[[1]])
```

---

## threshold_filter *Filter data frames with custom predicates*

---

### Description

**[Deprecated]** This function is deprecated and it's likely going to be dropped in the next release cycle.

Filter a single data frame or a list of data frames with custom predicates assembled from the function parameters.

## Usage

```
threshold_filter(x, threshold, cols_to_compare = "Value", comparators = ">")
```

## Arguments

| | |
|---|---|
| x | A data frame or a list of data frames |
| threshold | A numeric/integer vector or a named list of numeric/integer vectors |
| cols_to_compare | A character vector or a named list of character vectors |
| comparators | A character vector or a named list of character vectors. Must be one of the allowed values between `c("<", ">", "==", "!=", ">=", "<=")` |

## Value

A data frame or a list of data frames

## See Also

Other Data cleaning and pre-processing: `aggregate_metadata()`, `aggregate_values_by_key()`, `compute_near_integrations()`, `default_meta_agg()`, `outlier_filter()`, `outliers_by_pool_fragments()`, `purity_filter()`, `realign_after_collisions()`, `remove_collisions()`

## Examples

```
## Not run:
example_df <- tibble::tibble(
    a = c(20, 30, 40),
    b = c(40, 50, 60),
    c = c("a", "b", "c"),
    d = c(3L, 4L, 5L)
)
example_list <- list(
    first = example_df,
    second = example_df,
    third = example_df
)

filtered <- threshold_filter(example_list,
    threshold = list(
        first = c(20, 60),
        third = c(25)
    ),
    cols_to_compare = list(
        first = c("a", "b"),
        third = c("a")
    ),
    comparators = list(
        first = c(">", "<"),
        third = c(">=")
    )
```

```
)
filtered

## End(Not run)
```

---

`top_abund_tableGrob` *Summary top abundant tableGrobs for plots.*

---

## Description

Produce summary tableGrobs as R graphics. For this functionality the suggested package gridExtra is required. To visualize the resulting object:

```
gridExtra::grid.arrange(tableGrob)
```

## Usage

```
top_abund_tableGrob(
  df,
  id_cols = mandatory_IS_vars(),
  quant_col = "fragmentEstimate_sum_PercAbundance",
  by = "TimePoint",
  alluvial_plot = NULL,
  top_n = 10,
  tbl_cols = "GeneName",
  include_id_cols = FALSE,
  digits = 2,
  perc_symbol = TRUE,
  transform_by = NULL
)
```

## Arguments

| | |
|---|---|
| `df` | A data frame |
| `id_cols` | Character vector of id column names. To plot after alluvial, these columns must be the same as the `alluvia` argument of integration_alluvial_plot. |
| `quant_col` | Column name holding the quantification value. To plot after alluvial, these columns must be the same as the `plot_y` argument of integration_alluvial_plot. |
| `by` | The column name to subdivide tables for. The function will produce one table for each distinct value in `by`. To plot after alluvial, these columns must be the same as the `plot_x` argument of integration_alluvial_plot. |
| `alluvial_plot` | Either NULL or an alluvial plot for color mapping between values of y. |
| `top_n` | Integer. How many rows should the table contain at most? |
| `tbl_cols` | Table columns to show in the final output besides `quant_col`. |
| `include_id_cols` | Logical. Include `id_cols` in the output? |

| | |
|---|---|
| digits | Integer. Digits to show for the quantification column |
| perc_symbol | Logical. Show percentage symbol in the quantification column? |
| transform_by | Either a function or a purrr-style lambda. This function is applied to the column by before separating columns. If NULL no function is applied. Useful to modify column order in final table. |

## Value

A tableGrob object

## See Also

Other Plotting functions: CIS_volcano_plot(), HSC_population_plot(), circos_genomic_density(), fisher_scatterplot(), integration_alluvial_plot(), sharing_heatmap(), sharing_venn(), top_cis_overtime_heatmap()

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
abund <- compute_abundance(x = aggreg)
grob <- top_abund_tableGrob(abund)
gridExtra::grid.arrange(grob)

# with transform
grob <- top_abund_tableGrob(abund, transform_by = ~ as.numeric(.x))
```

---

# top_cis_overtime_heatmap

*Heatmaps for the top N common insertion sites over time.*

---

## Description

**[Experimental]** This function computes the visualization of the results of the function CIS_grubbs_overtime() in the form of heatmaps for the top N selected genes over time.

## Usage

```
top_cis_overtime_heatmap(
  x,
  n_genes = 20,
  timepoint_col = "TimePoint",
  group_col = "group",
```

```
  onco_db_file = "proto_oncogenes",
  tumor_suppressors_db_file = "tumor_suppressors",
  species = "human",
  known_onco = known_clinical_oncogenes(),
  suspicious_genes = clinical_relevant_suspicious_genes(),
  significance_threshold = 0.05,
  plot_values = c("minus_log_p", "p"),
  p_value_correction = c("fdr", "bonferroni"),
  prune_tp_treshold = 20,
 gene_selection_param = c("trimmed", "n", "mean", "sd", "median", "mad", "min", "max"),
  fill_0_selection = TRUE,
  fill_NA_in_heatmap = FALSE,
  heatmap_color_palette = "default",
  title_generator = NULL,
  save_as_files = FALSE,
  files_format = c("pdf", "png", "tiff", "bmp", "jpg"),
  folder_path = NULL,
  ...
)
```

## Arguments

| | |
|---|---|
| x | Output of the function CIS_grubbs_overtime(), either in single data frame form or nested lists |
| n_genes | Number of top genes to consider |
| timepoint_col | The name of the time point column in x |
| group_col | The name of the group column in x |
| onco_db_file | Uniprot file for proto-oncogenes (see details). If different from default, should be supplied as a path to a file. |
| tumor_suppressors_db_file | Uniprot file for tumor-suppressor genes. If different from default, should be supplied as a path to a file. |
| species | One between "human", "mouse" and "all" |
| known_onco | Data frame with known oncogenes. See details. |
| suspicious_genes | Data frame with clinical relevant suspicious genes. See details. |
| significance_threshold | The significance threshold |
| plot_values | Which kind of values should be plotted? Can either be "p" for the p-value or "minus_log_p" for a scaled p-value of the Grubbs test |
| p_value_correction | One among "bonferroni" and "fdr" |
| prune_tp_treshold | Minimum number of genes to retain a time point. See details. |

gene_selection_param
: The descriptive statistic measure to decide which genes to plot, possible choices are "trimmed", "n", "mean", "sd", "median","mad", "min", "max". See details.

fill_0_selection
: Fill NA values with 0s before computing statistics for each gene? (TRUE/FALSE)

fill_NA_in_heatmap
: Fill NA values with 0 when plotting the heatmap? (TRUE/FALSE)

heatmap_color_palette
: Colors for values in the heatmaps, either "default" or a function producing a color palette, obtainable via grDevices::colorRampPalette.

title_generator
: Either NULL or a function. See details.

save_as_files
: Should heatmaps be saved to files on disk? (TRUE/FALSE)

files_format
: The extension of the files produced, supported formats are "pdf", "png", "tiff", "bmp", "jpg". Relevant only if files_format = TRUE

folder_path
: Path to the folder where files will be saved

...
: Other params to pass to pheatmap::pheatmap

## Details

**Oncogene and tumor suppressor genes files:**

These files are included in the package for user convenience and are simply UniProt files with gene annotations for human and mouse. For more details on how this files were generated use the help ?tumor_suppressors, ?proto_oncogenes

**Known oncogenes:**

The default values are included in this package and it can be accessed by doing:

```
known_clinical_oncogenes()
```

If the user wants to change this parameter the input data frame must preserve the column structure. The same goes for the suspicious_genes parameter (DOIReference column is optional):

```
clinical_relevant_suspicious_genes()
```

**Top N gene selection:**

Since the genes present in different time point slices are likely different, the decision process to select the final top N genes to represent in the heatmap follows this logic:

- Each time point slice is arranged either in ascending order (if we want to plot the p-value) or in descending order (if we want to plot the scaled p-value) and the top n genes are selected
- A series of statistics are computed over the union set of genes on ALL time points (min, max, mean, ...)
- A decision is taken by considering the ordered gene_selection_param (order depends once again if the values are scaled or not), and the first N genes are selected for plotting.

*Filling NA values prior calculations:*
It is possible to fill NA values (aka missing combinations of GENE/TP) with 0s prior computing the descriptive statistics on which gene selection is based. Please keep in mind that this has an impact on the final result, since for computing metrics such as the mean, NA values are usually removed, decreasing the overall number of values considered - this does not hold when NA values are substituted with 0s.

*The statistics:*
Statistics are computed for each gene over all time points of each group. More in detail, `n`: counts the number of instances (rows) in which the genes appears, aka it counts the time points in which the gene is present. NOTE: if `fill_0_selection` option is set to `TRUE` this value will be equal for all genes! All other statistics as per the argument `gene_selection_param` map to the corresponding R functions with the exception of `trimmed` which is a simple call to the `mean` function with the argument `trimmed = 0.1`.

**Aesthetics:**

It is possible to customise the appearence of the plot through different parameters.

- `fill_NA_in_heatmap` tells the function whether missing combinations of GENE/TP should be plotted as NA or filled with a value (1 if p-value, 0 if scaled p-value)
- A title generator function can be provided to dynamically create a title for the plots: the function can accept two positional arguments for the group identifier and the number of selected genes respectively. If one or none of the arguments are of interest, they can be absorbed with `...`.
- `heatmap_color_palette` can be used to specify a function from which colors are sampled (refers to the colors of values only)
- To change the colors associated with annotations instead, use the argument `annotation_colors` of `pheatmap::pheatmap()` - it must be set to a list with this format:

```
list(
  KnownGeneClass = c("OncoGene" = color_spec,
                     "Other" = color_spec,
                     "TumSuppressor" = color_spec),
  ClinicalRelevance = c("TRUE" = color_spec,
                        "FALSE" = color_spec),
  CriticalForInsMut = c("TRUE" = color_spec,
                        "FALSE" = color_spec)
)
```

## Value

Either a list of graphical objects or a list of paths where plots were saved

## See Also

Other Plotting functions: `CIS_volcano_plot()`, `HSC_population_plot()`, `circos_genomic_density()`, `fisher_scatterplot()`, `integration_alluvial_plot()`, `sharing_heatmap()`, `sharing_venn()`, `top_abund_tableGrob()`

## Examples

```
data("integration_matrices", package = "ISAnalytics")
data("association_file", package = "ISAnalytics")
aggreg <- aggregate_values_by_key(
    x = integration_matrices,
    association_file = association_file,
    value_cols = c("seqCount", "fragmentEstimate")
)
cis_overtime <- CIS_grubbs_overtime(aggreg)
hmaps <- top_cis_overtime_heatmap(cis_overtime$cis,
    fill_NA_in_heatmap = TRUE
)

# To re-plot:
# grid::grid.newpage()
# grid::grid.draw(hmaps$PT001$gtable)
```

---

# top_integrations *Sorts and keeps the top n integration sites based on the values in a given column.*

---

## Description

**[Stable]** The input data frame will be sorted by the highest values in the columns specified and the top n rows will be returned as output. The user can choose to keep additional columns in the output by passing a vector of column names or passing 2 "shortcuts":

- `keep = "everything"` keeps all columns in the original data frame
- `keep = "nothing"` only keeps the mandatory columns (`mandatory_IS_vars()`) plus the columns in the `columns` parameter.

## Usage

```
top_integrations(
  x,
  n = 20,
  columns = "fragmentEstimate_sum_RelAbundance",
  keep = "everything",
  key = NULL
)
```

## Arguments

| | |
|---|---|
| `x` | An integration matrix (data frame containing `mandatory_IS_vars()`) |
| `n` | How many integrations should be sliced (in total or for each group)? Must be numeric or integer and greater than 0 |
| `columns` | Columns to use for the sorting. If more than a column is supplied primary ordering is done on the first column, secondary ordering on all other columns |

| | |
|---|---|
| keep | Names of the columns to keep besides mandatory_IS_vars() and columns |
| key | Either NULL or a character vector of column names to group by. If not NULL the input will be grouped and the top fraction will be extracted from each group. |

## Value

Either a data frame with at most n rows or a data frames with at most n*(number of groups) rows.

## Required tags

The function will explicitly check for the presence of these tags:

- All columns declared in mandatory_IS_vars()

## See Also

Other Analysis functions: CIS_grubbs(), HSC_population_size_estimate(), compute_abundance(), cumulative_is(), gene_frequency_fisher(), is_sharing(), iss_source(), sample_statistics(), top_targeted_genes()

## Examples

```
smpl <- tibble::tibble(
    chr = c("1", "2", "3", "4", "5", "6"),
    integration_locus = c(14536, 14544, 14512, 14236, 14522, 14566),
    strand = c("+", "+", "-", "+", "-", "+"),
    CompleteAmplificationID = c("ID1", "ID2", "ID1", "ID1", "ID3", "ID2"),
    Value = c(3, 10, 40, 2, 15, 150),
    Value2 = c(456, 87, 87, 9, 64, 96),
    Value3 = c("a", "b", "c", "d", "e", "f")
)
top <- top_integrations(smpl,
    n = 3,
    columns = c("Value", "Value2"),
    keep = "nothing"
)
top_key <- top_integrations(smpl,
    n = 3,
    columns = "Value",
    keep = "Value2",
    key = "CompleteAmplificationID"
)
```

---

# top_targeted_genes *Top n targeted genes based on number of IS.*

---

## Description

**[Experimental]** Produces a summary of the number of integration events per gene, orders the table in decreasing order and slices the first n rows - either on all the data frame or by group.

## Usage

```
top_targeted_genes(
  x,
  n = 20,
  key = c("SubjectID", "CellMarker", "Tissue", "TimePoint"),
  consider_chr = TRUE,
  consider_gene_strand = TRUE,
  as_df = TRUE
)
```

## Arguments

| | |
|---|---|
| x | An integration matrix - must be annotated |
| n | Number of rows to slice |
| key | If slice has to be performed for each group, the character vector of column names that identify the groups. If NULL considers the whole input data frame. |
| consider_chr | Logical, should the chromosome be taken into account? See details. |
| consider_gene_strand | Logical, should the gene strand be taken into account? See details. |
| as_df | If computation is performed by group, TRUE returns all groups merged in a single data frame with a column containing the group id. If FALSE returns a named list. |

## Details

### Gene grouping:

When producing a summary of IS by gene, there are different options that can be chosen. The argument consider_chr accounts for the fact that some genes (same gene symbol) may span more than one chromosome: if set to TRUE counts of IS will be separated for those genes that span 2 or more chromosomes - in other words they will be in 2 different rows of the output table. On the contrary, if the argument is set to FALSE, counts will be produced in a single row.

NOTE: the function counts **DISTINCT** integration events, which logically corresponds to a union of sets. Be aware of the fact that counts per group and counts with different arguments might be different: if for example counts are performed by considering chromosome and there is one gene symbol with 2 different counts, the sum of those 2 will likely not be equal to the count obtained by performing the calculations without considering the chromosome.

The same reasoning can be applied for the argument consider_gene_strand, that takes into account the strand of the gene.

## Value

A data frame or a list of data frames

## Required tags

The function will explicitly check for the presence of these tags:

- chromosome

- locus
- gene_symbol
- gene_strand

Note that the tags "gene_strand" and "chromosome" are explicitly required only if `consider_chr = TRUE` and/or `consider_gene_strand = TRUE`.

## See Also

Other Analysis functions: `CIS_grubbs()`, `HSC_population_size_estimate()`, `compute_abundance()`, `cumulative_is()`, `gene_frequency_fisher()`, `is_sharing()`, `iss_source()`, `sample_statistics()`, `top_integrations()`

## Examples

```
data("integration_matrices", package = "ISAnalytics")
top_targ <- top_targeted_genes(
    integration_matrices,
    key = NULL
)
top_targ
```

---

`transform_columns` *Apply transformations to an arbitrary number of columns.*

---

## Description

This function takes a named list of purr-style lambdas where names are the names of the columns in the data frame that must be transformed. NOTE: the columns are overridden, not appended.

## Usage

```
transform_columns(df, transf_list)
```

## Arguments

| | |
|---|---|
| `df` | The data frame on which transformations should be operated |
| `transf_list` | A named list of purrr-style lambdas, where names are column names the function should be applied to. |

## Details

Lambdas provided in input must be transformations, aka functions that take in input a vector and return a vector of the same length as the input.

If the input transformation list contains column names that are not present in the input data frame, they are simply ignored.

## Value

A data frame with transformed columns

## See Also

Other Utilities: as_sparse_matrix(), comparison_matrix(), enable_progress_bars(), export_ISA_settings(), generate_Vispa2_launch_AF(), generate_blank_association_file(), generate_default_folder_structure(), import_ISA_settings(), separate_quant_matrices()

## Examples

```
df <- tibble::tribble(
    ~A, ~B, ~C, ~D,
    1, 2, "a", "aa",
    3, 4, "b", "bb",
    5, 6, "c", "cc"
)
lambdas <- list(A = ~ .x + 1, B = ~ .x + 2, C = ~ stringr::str_to_upper(.x))
transform_columns(df, lambdas)
```

---

# unzip_file_system *A utility function to unzip and use example file systems included in the package*

---

## Description

**[Deprecated]** From ISAnalytics 1.5.4 this function is defunct, since the package doesn't include example tabular files anymore. Use the function generate_default_folder_structure() to generate a default folder structure for running tests and play with the package import functions. If you don't need to test import functions, you can simply load package included data via data("integration_matrices") or data("association_file").

## Usage

```
unzip_file_system(zipfile, name)
```

## Arguments

| | |
|---|---|
| zipfile | The zipped file to decompress |
| name | The name of the folder in the zipped archive ("fs" or "fserr") |

## Value

A path to reference

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