

Package ‘scPipe’

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Title pipeline for single cell RNA-seq data analysis

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Maintainer Luyi Tian <tian.l@wehi.edu.au>

Author Luyi Tian

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GeneExpression, SingleCell, Visualization, SequenceMatching,
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Description A preprocessing pipeline for single cell RNA-seq data that starts from the fastq files and produces a gene count matrix with associated quality control information. It can process fastq data generated by CEL-seq, MARS-seq, Drop-seq, Chromium 10x and SMART-seq protocols.

Depends R (>= 3.4), ggplot2, methods, SingleCellExperiment

LinkingTo Rcpp, Rhtslib (>= 1.13.1), zlibbioc, testthat

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reshape, BiocGenerics, robustbase, scales, utils, stats,
S4Vectors, SummarizedExperiment, AnnotationDbi, org.Hs.eg.db,
org.Mm.eg.db, stringr, rtracklayer, hashmap, dplyr,
GenomicRanges, magrittr, glue

SystemRequirements C++11, GNU make

License GPL (>= 2)

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NeedsCompilation yes

URL <https://github.com/LuyiTian/scPipe>

BugReports <https://github.com/LuyiTian/scPipe>

Suggests Rsubread, knitr, rmarkdown, testthat

VignetteBuilder knitr

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.qq_outliers_robust *Detect outliers based on robust linear regression of QQ plot*

Description

Detect outliers based on robust linear regression of QQ plot

Usage

```
.qq_outliers_robust(x, df, conf)
```

Arguments

| | |
|------|-----------------------------------------------|
| x | a vector of mahalanobis distance |
| df | degree of freedom for chi-square distribution |
| conf | confidence for linear regression |

Value

cell names of outliers

| | |
|-------------|-------------------------------|
| anno_import | <i>Import gene annotation</i> |
|-------------|-------------------------------|

Description

Because of the variations in data format depending on annotation source, this function has only been tested with human annotation from ENSEMBL, RefSeq and Gencode. If it behaves unexpectedly with any annotation please submit an issue at www.github.com/LuyiTian/scPipe with details.

Usage

```
anno_import(filename)
```

Arguments

| | |
|----------|-------------------------------------------------------------------|
| filename | The name of the annotation gff3 or gtf file. File can be gzipped. |
|----------|-------------------------------------------------------------------|

Details

Imports and GFF3 or GTF gene annotation file and transforms it into a SAF formatted data.frame. SAF described at <http://bioinf.wehi.edu.au/featureCounts/>. SAF contains positions for exons, strand and the GeneID they are associated with.

Value

data.frame containing exon information in SAF format

Examples

```
ens_chrY <- anno_import(system.file("extdata", "ensembl_hg38_chrY.gtf.gz", package = "scPipe"))
```

| | |
|-------------|--------------------------------------------------------------------------------|
| anno_to_saf | <i>Convert annotation from GenomicRanges to Simple Annotation Format (SAF)</i> |
|-------------|--------------------------------------------------------------------------------|

Description

This function converts a GRanges object into a data.frame of the SAF format for scPipe's consumption. The GRanges object should contain a "type" column where at least some features are annotated as "exon", in addition there should be a gene_id column specifying the gene to which the exon belongs. In the SAF only the gene ID, chromosome, start, end and strand are recorded, this is a gene-exon centric format, with all entries containing the same gene ID treated as exons of that gene. It is possible to count alternative features by setting the gene_id column to an arbitrary feature name and having alternative features in the SAF table, the main caveat is that the features are still treated as exons, and the mapping statistics for exon and intron will not reflect biological exons and introns but rather the annotation features.

Usage

```
anno_to_saf(anno)
```

Arguments

anno The GRanges object containing exon information

Details

Convert a GRanges object containing type and gene_id information into a SAF format data.frame. SAF described at <http://bioinf.wehi.edu.au/featureCounts/>. SAF contains positions for exons, strand and the GeneID they are associated with.

Value

data.frame containing exon information in SAF format

Examples

```
anno <- system.file("extdata", "ensembl_hg38_chrY.gtf.gz", package = "scPipe")
saf_chrY <- anno_to_saf(rtracklayer::import(anno))
```

| | |
|----------------------|----------------------------------------------------|
| calculate_QC_metrics | <i>Calculate QC metrics from gene count matrix</i> |
|----------------------|----------------------------------------------------|

Description

Calculate QC metrics from gene count matrix

Usage

```
calculate_QC_metrics(sce)
```

Arguments

sce a SingleCellExperiment object containing gene counts

Details

get QC metrics using gene count matrix. The QC statistics added are

- number_of_genes number of genes detected.
- total_count_per_cell sum of read number after UMI deduplication.
- non_mt_percent 1 - percentage of mitochondrial gene counts. Mitochondrial genes are retrieved by GO term GO:0005739
- non_ERCC_percent ratio of exon counts to ERCC counts
- non_ribo_percent 1 - percentage of ribosomal gene counts ribosomal genes are retrieved by GO term GO:0005840.

Value

an SingleCellExperiment with updated QC metrics

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

# The sample qc data already run through function `calculate_QC_metrics`.
# So we delete these columns and run `calculate_QC_metrics` to get them again:
colnames(colnames(QC_metrics(sce)))
QC_metrics(sce) = QC_metrics(sce)[,c("unaligned", "aligned_unmapped", "mapped_to_exon")]
sce = calculate_QC_metrics(sce)
colnames(QC_metrics(sce))
```

cell_barcode_matching *cell barcode demultiplex statistics for a small sample scRNA-seq dataset to demonstrate capabilities of scPipe*

Description

This data.frame contains cell barcode demultiplex statistics with several rows:

- barcode_unmatch_ambiguous_mapping is the number of reads that do not match any barcode, but aligned to the genome and mapped to multiple features.
- barcode_unmatch_mapped_to_intron is the number of reads that do not match any barcode, but aligned to the genome and mapped to intron.
- barcode_match is the number of reads that match the cell barcodes

- barcode_unmatch_unaligned is the number of reads that do not match any barcode, and not aligned to the genome
- barcode_unmatch_aligned is the number of reads that do not match any barcode, but aligned to the genome and do not mapped to any feature
- barcode_unmatch_mapped_to_exon is the number of reads that do not match any barcode, but aligned to the genome and mapped to the exon

Usage

```
sc_sample_qc
```

Format

a data.frame instance, one row per cell.

Value

NULL, but makes a data frame with cell barcode demultiplex statistics

Author(s)

Luyi Tian

Source

Christin Biben (WEHI). She FACS sorted cells from several immune cell types including B cells, granulocyte and some early progenitors.

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

demultiplex_info(sce)
```

convert_geneid

convert the gene ids of a SingleCellExperiment object

Description

convert the gene ids of a SingleCellExperiment object

Usage

```
convert_geneid(sce, returns = "external_gene_name", all = TRUE)
```

Arguments

| | |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| sce | a SingleCellExperiment object |
| returns | the gene id which is set as return. Default to be 'external_gene_name'. A possible list of attributes can be retrieved using the function <code>listAttributes</code> from <code>biomaRt</code> package. The commonly used id types are 'external_gene_name', 'ensembl_gene_id' or 'entrezgene'. |
| all | logic. For genes that cannot convert to new gene id, keep them with the old id or delete them. The default is keep them. |

Details

convert the gene id of all datas in the SingleCellExperiment object

Value

sce with converted id

Examples

```
# the gene id in example data are `external_gene_name`
# the following example will convert it to `external_gene_name`.
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication
head(rownames(sce))
sce = convert_geneid(sce, return="external_gene_name")
head(rownames(sce))
```

```
create_processed_report
```

```
create_processed_report
```

Description

create an HTML report summarising pro-processed data. This is an alternative to the more verbose `create_report` that requires only the processed counts and stats folders.

Usage

```
create_processed_report(outdir = ".", organism, gene_id_type,
  report_name = "report")
```

Arguments

| | |
|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| outdir | output folder |
| organism | the organism of the data. List of possible names can be retrieved using the function 'listDatasets' from 'biomaRt' package. (i.e 'mmusculus_gene_ensembl' or 'hsapiens_gene_ensembl') |
| gene_id_type | gene id type of the data A possible list of ids can be retrieved using the function 'listAttributes' from 'biomaRt' package. the commonly used id types are 'external_gene_name', 'ensembl_gene_id' or 'entrezgene' |
| report_name | the name of the report .Rmd and .html files. |

Examples

```
## Not run:
create_report(
  outdir="output_dir_of_scPipe",
  organism="mmusculus_gene_ensembl",
  gene_id_type="ensembl_gene_id")

## End(Not run)
```

| | |
|---------------|----------------------|
| create_report | <i>create_report</i> |
|---------------|----------------------|

Description

create an HTML report using data generated by preprocessing step.

Usage

```
create_report(sample_name, outdir, r1 = "NA", r2 = "NA",
  outfq = "NA", read_structure = list(bs1 = 0, b11 = 0, bs2 = 0, b12 =
  0, us = 0, ul = 0), filter_settings = list(rmlow = TRUE, rmN = TRUE,
  minq = 20, numbq = 2), align_bam = "NA", genome_index = "NA",
  map_bam = "NA", exon_anno = "NA", stnd = TRUE, fix_chr = FALSE,
  barcode_anno = "NA", max_mis = 1, UMI_cor = 1, gene_fl = FALSE,
  organism, gene_id_type)
```

Arguments

| | |
|-----------------|------------------------------------------------------------------------------------|
| sample_name | sample name |
| outdir | output folder |
| r1 | file path of read1 |
| r2 | file path of read2 default to be NULL |
| outfq | file path of the output of sc_trim_barcode |
| read_structure | a list contains read structure configuration. For more help see '?sc_trim_barcode' |
| filter_settings | a list contains read filter settings for more help see '?sc_trim_barcode' |
| align_bam | the aligned bam file |

| | |
|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| genome_index | genome index used for alignment |
| map_bam | the mapped bam file |
| exon_anno | the gff exon annotation used. Can have multiple files |
| std | whether to perform strand specific mapping |
| fix_chr | add 'chr' to chromosome names, fix inconsistent names. |
| barcode_anno | cell barcode annotation file path. |
| max_mis | maximum mismatch allowed in barcode. Default to be 1 |
| UMI_cor | correct UMI sequence error: 0 means no correction, 1 means simple correction and merge UMI with distance 1. |
| gene_fl | whether to remove low abundant gene count. Low abundant is defined as only one copy of one UMI for this gene |
| organism | the organism of the data. List of possible names can be retrieved using the function 'listDatasets' from 'biomaRt' package. (i.e 'mmusculus_gene_ensembl' or 'hsapiens_gene_ensembl') |
| gene_id_type | gene id type of the data A possible list of ids can be retrieved using the function 'listAttributes' from 'biomaRt' package. the commonly used id types are 'external_gene_name', 'ensembl_gene_id' or 'entrezgene' |

Value

no return

Examples

```
## Not run:
create_report(sample_name="sample_001",
  outdir="output_dir_of_scPipe",
  r1="read1.fq",
  r2="read2.fq",
  outfq="trim.fq",
  read_structure=list(bs1=-1, b1=2, bs2=6, b2=8, us=0, ul=6),
  filter_settings=list(rmlow=TRUE, rmN=TRUE, minq=20, numq=2),
  align_bam="align.bam",
  genome_index="mouse.index",
  map_bam="aligned.mapped.bam",
  exon_anno="exon_anno.gff3",
  std=TRUE,
  fix_chr=FALSE,
  barcode_anno="cell_barcode.csv",
  max_mis=1,
  UMI_cor=1,
  gene_fl=FALSE,
  organism="mmusculus_gene_ensembl",
  gene_id_type="ensembl_gene_id")

## End(Not run)
```

| | |
|-------------------|----------------------------------------------------------------------------------------------|
| create_sce_by_dir | <i>create a SingleCellExperiment object from data folder generated by preprocessing step</i> |
|-------------------|----------------------------------------------------------------------------------------------|

Description

after we run `sc_gene_counting` and finish the preprocessing step. `create_sce_by_dir` can be used to generate the `SingleCellExperiment` object from the folder that contains gene count matrix and QC statistics. it can also generate the html report based on the gene count and quality control statistics

Usage

```
create_sce_by_dir(datadir, organism = NULL, gene_id_type = NULL,
  pheno_data = NULL, report = FALSE)
```

Arguments

| | |
|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>datadir</code> | the directory that contains all the data and 'stat' subfolder. |
| <code>organism</code> | the organism of the data. List of possible names can be retrieved using the function 'listDatasets' from 'biomaRt' package. (i.e 'mmusculus_gene_ensembl' or 'hsapiens_gene_ensembl') |
| <code>gene_id_type</code> | gene id type of the data A possible list of ids can be retrieved using the function 'listAttributes' from 'biomaRt' package. the commonly used id types are 'external_gene_name', 'ensembl_gene_id' or 'entrezgene' |
| <code>pheno_data</code> | the external phenotype data that linked to each single cell. This should be an AnnotatedDataFrame object |
| <code>report</code> | whether to generate the html report in the data folder |

Details

after we run `sc_gene_counting` and finish the preprocessing step. `create_sce_by_dir` can be used to generate the `SingleCellExperiment` object from the folder that contains gene count matrix and QC statistics.

Value

a `SingleCellExperiment` object

Examples

```
## Not run:
# the sce can be created from the output folder of scPipe
# please refer to the vignettes
sce = create_sce_by_dir(datadir="output_dir_of_scPipe",
  organism="mmusculus_gene_ensembl",
  gene_id_type="ensembl_gene_id")

## End(Not run)
# or directly from the gene count and quality control matrix:
data("sc_sample_data")
```

```
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication
dim(sce)
```

demultiplex_info *demultiplex_info*

Description

Get or set cell barcode demultiplex results in a `SingleCellExperiment` object

Usage

```
demultiplex_info(object)

demultiplex_info(object) <- value

demultiplex_info.sce(object)

## S4 method for signature 'SingleCellExperiment'
demultiplex_info(object)

## S4 replacement method for signature 'SingleCellExperiment'
demultiplex_info(object) <- value
```

Arguments

`object` A `SingleCellExperiment` object.
`value` Value to be assigned to corresponding object.

Value

a dataframe of cell barcode demultiplex information
A `DataFrame` of cell barcode demultiplex results.

Author(s)

Luyi Tian

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
```

```

QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

demultiplex_info(sce)

```

detect_outlier

Detect outliers based on QC metrics

Description

This algorithm will try to find comp number of components in quality control metrics using a Gaussian mixture model. Outlier detection is performed on the component with the most genes detected. The rest of the components will be considered poor quality cells. More cells will be classified low quality as you increase comp.

Usage

```

detect_outlier(sce, comp = 1, sel_col = NULL, type = c("low", "both",
"high"), conf = c(0.9, 0.99), batch = FALSE)

```

Arguments

| | |
|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| sce | a SingleCellExperiment object containing QC metrics. |
| comp | the number of component used in GMM. Depending on the quality of the experiment. |
| sel_col | a vector of column names which indicate the columns to use for QC. By default it will be the statistics generated by 'calculate_QC_metrics()' |
| type | only looking at low quality cells ('low') or possible doublets ('high') or both ('both') |
| conf | confidence interval for linear regression at lower and upper tails. Usually, this is smaller for lower tail because we hope to pick out more low quality cells than doublets. |
| batch | whether to perform quality control separately for each batch. Default is FALSE. If set to TRUE then you should have a column called 'batch' in the 'colData(sce)'. |

Details

detect outlier using Mahalanobis distances

Value

an updated SingleCellExperiment object with an 'outlier' column in colData

Examples

```

data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication
# the sample qc data already run through function `calculate_QC_metrics`
# for a new sce please run `calculate_QC_metrics` before `detect_outlier`
sce = detect_outlier(sce)
table(QC_metrics(sce)$outliers)

```

gene_id_type

Get or set gene_id_type from a SingleCellExperiment object

Description

Get or set gene_id_type from a SingleCellExperiment object

Usage

```

gene_id_type(object)

gene_id_type(object) <- value

gene_id_type.sce(object)

## S4 method for signature 'SingleCellExperiment'
gene_id_type(object)

## S4 replacement method for signature 'SingleCellExperiment'
gene_id_type(object) <- value

```

Arguments

object A [SingleCellExperiment](#) object.
value Value to be assigned to corresponding object.

Value

the gene id type used by Biomart
gene id type string

Author(s)

Luyi Tian

Examples

```

data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

gene_id_type(sce)

```

| | |
|---------------|----------------------------------|
| get_ercc_anno | <i>Get ERCC annotation table</i> |
|---------------|----------------------------------|

Description

Helper function to retrieve ERCC annotation as a dataframe in SAF format

Usage

```
get_ercc_anno()
```

Value

data.frame containing ERCC annotation

Examples

```
ercc_anno <- get_ercc_anno()
```

| | |
|-----------------|--------------------------------------------------------------------|
| get_genes_by_GO | <i>Get genes related to certain GO terms from biomart database</i> |
|-----------------|--------------------------------------------------------------------|

Description

Get genes related to certain GO terms from biomart database

Usage

```
get_genes_by_GO(returns = "ensembl_gene_id",
  dataset = "mmusculus_gene_ensembl", go = NULL)
```

Arguments

| | |
|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| returns | the gene id which is set as return. Default to be ensembl id A possible list of attributes can be retrieved using the function <code>listAttributes</code> from <code>biomaRt</code> package. The commonly used id types are 'external_gene_name', 'ensembl_gene_id' or 'entrezgene'. |
| dataset | Dataset you want to use. List of possible datasets can be retrieved using the function <code>listDatasets</code> from <code>biomaRt</code> package. |
| go | a vector of GO terms |

Details

Get genes related to certain GO terms from biomart database

Value

a vector of gene ids.

Examples

```
# get all genes under GO term GO:0005739 in mouse, return ensembl gene id
get_genes_by_GO(returns="ensembl_gene_id",
  dataset="mmusculus_gene_ensembl",
  go=c('GO:0005739'))
```

get_read_str

Get read structure for particular scRNA-seq protocol

Description

The supported protocols are:

- CelSeq
- CelSeq2
- DropSeq
- 10x (also called ChromiumV1)

If you know the structure of a specific protocol and would like it supported, please leave a issue post at www.github.com/luyitian/scPipe.

Usage

```
get_read_str(protocol)
```

Arguments

| | |
|----------|----------------------|
| protocol | name of the protocol |
|----------|----------------------|

Value

list of UMI and Barcode locations for use in other scPipe functions

Examples

```
get_read_str("celseq")
```

| | |
|--------------|---------------------------------------------------------------|
| organism.sce | <i>Get or set organism from a SingleCellExperiment object</i> |
|--------------|---------------------------------------------------------------|

Description

Get or set organism from a SingleCellExperiment object

Usage

```
organism.sce(object)

## S4 method for signature 'SingleCellExperiment'
organism(object)

## S4 replacement method for signature 'SingleCellExperiment'
organism(object) <- value
```

Arguments

| | |
|--------|------------------------------------------------|
| object | A SingleCellExperiment object. |
| value | Value to be assigned to corresponding object. |

Value

organism string

Author(s)

Luyi Tian

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

organism(sce)
```

| | |
|------------------|-------------------------|
| plot_demultiplex | <i>plot_demultiplex</i> |
|------------------|-------------------------|

Description

Plot cell barcode demultiplexing result for the SingleCellExperiment. The barcode demultiplexing result is shown using a barplot, with the bars indicating proportions of total reads. Barcode matches and mismatches are summarised along with whether or not the read mapped to the genome. High proportion of genome aligned reads with no barcode match may indicate barcode integration failure.

Usage

```
plot_demultiplex(sce)
```

Arguments

sce a SingleCellExperiment object

Value

a ggplot2 bar chart

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

plot_demultiplex(sce)
```

| | |
|--------------|-----------------------------------------------------------------|
| plot_mapping | <i>Plot mapping statistics for SingleCellExperiment object.</i> |
|--------------|-----------------------------------------------------------------|

Description

Plot mapping statistics for SingleCellExperiment object.

Usage

```
plot_mapping(sce, sel_col = NULL, percentage = FALSE, dataname = "")
```

Arguments

| | |
|------------|-------------------------------------------------------------------------------------------------------------|
| sce | a SingleCellExperiment object |
| sel_col | a vector of column names, indicating the columns to use for plot. by default it will be the mapping result. |
| percentage | TRUE to convert the number of reads to percentage |
| dataname | the name of this dataset, used as plot title |

Value

a ggplot2 object

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

plot_mapping(sce,percentage=TRUE,dataname="sc_sample")
```

| | |
|---------------|---------------------------------------------------------------------------------|
| plot_QC_pairs | <i>Plot GGally pairs plot of QC statistics from SingleCellExperiment object</i> |
|---------------|---------------------------------------------------------------------------------|

Description

Plot GGally pairs plot of QC statistics from SingleCellExperiment object

Usage

```
plot_QC_pairs(sce, sel_col = NULL)
```

Arguments

| | |
|---------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| sce | a SingleCellExperiment object |
| sel_col | a vector of column names which indicate the columns to use for plot. By default it will be the statistics generated by ‘calculate_QC_metrics()’ |

Value

a ggplot2 object

Examples

```

data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication
sce = detect_outlier(sce)

plot_QC_pairs(sce)

```

| | |
|--------------|---------------------------------------|
| plot_UMI_dup | <i>Plot UMI duplication frequency</i> |
|--------------|---------------------------------------|

Description

Plot the UMI duplication frequency.

Usage

```
plot_UMI_dup(sce, log10_x = TRUE)
```

Arguments

| | |
|---------|---------------------------------------|
| sce | a SingleCellExperiment object |
| log10_x | whether to use log10 scale for x axis |

Value

a line chart of the UMI duplication frequency

Examples

```

data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

plot_UMI_dup(sce)

```

`QC_metrics`*Get or set quality control metrics in a SingleCellExperiment object*

Description

Get or set quality control metrics in a SingleCellExperiment object

Usage

```
QC_metrics(object)

QC_metrics(object) <- value

QC_metrics.sce(object)

## S4 method for signature 'SingleCellExperiment'
QC_metrics(object)

## S4 replacement method for signature 'SingleCellExperiment'
QC_metrics(object) <- value
```

Arguments

| | |
|---------------------|------------------------------------------------|
| <code>object</code> | A SingleCellExperiment object. |
| <code>value</code> | Value to be assigned to corresponding object. |

Value

a dataframe of quality control metrics
A DataFrame of quality control metrics.

Author(s)

Luyi Tian

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
QC_metrics(sce) = sc_sample_qc

head(QC_metrics(sce))
```

| | |
|-----------------|------------------------------------------------|
| remove_outliers | <i>Remove outliers in SingleCellExperiment</i> |
|-----------------|------------------------------------------------|

Description

Removes outliers flagged by `detect_outliers()`

Usage

```
remove_outliers(sce)
```

Arguments

sce a SingleCellExperiment object

Value

a SingleCellExperiment object without outliers

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication
sce = detect_outlier(sce)
dim(sce)
sce = remove_outliers(sce)
dim(sce)
```

| | |
|--------|----------------------------------------------|
| scPipe | <i>scPipe - single cell RNA-seq pipeline</i> |
|--------|----------------------------------------------|

Description

The scPipe will do cell barcode demultiplexing, UMI deduplication and quality control on fastq data generated from all protocols

Author(s)

Luyi Tian <tian.l@wehi.edu.au>; Shian Su <su.s@wehi.edu.au>

sc_count_aligned_bam *sc_count_aligned_bam*

Description

Wrapper to run [sc_exon_mapping](#), [sc_demultiplex](#) and [sc_gene_counting](#) with a single command

Usage

```
sc_count_aligned_bam(inbam, outbam, annofn, bam_tags = list(am = "YE", ge
= "GE", bc = "BC", mb = "OX"), bc_len = 8, UMI_len = 6,
std = TRUE, fix_chr = FALSE, outdir, bc_anno, max_mis = 1,
mito = "MT", has_UMI = TRUE, UMI_cor = 1, gene_fl = FALSE,
keep_mapped_bam = TRUE, nthreads = 1)
```

Arguments

| | |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| inbam | input aligned bam file. can have multiple files as input |
| outbam | output bam filename |
| annofn | single string or vector of gff3 annotation filenames, data.frame in SAF format or GRanges object containing complete gene_id metadata column. |
| bam_tags | list defining BAM tags where mapping information is stored. <ul style="list-style-type: none"> • "am": mapping status tag • "ge": gene id • "bc": cell barcode tag • "mb": molecular barcode tag |
| bc_len | total barcode length |
| UMI_len | UMI length |
| std | TRUE to perform strand specific mapping. (default: TRUE) |
| fix_chr | TRUE to add 'chr' to chromosome names, MT to chrM. (default: FALSE) |
| outdir | output folder |
| bc_anno | barcode annotation, first column is cell id, second column is cell barcode sequence |
| max_mis | maximum mismatch allowed in barcode. (default: 1) |
| mito | mitochondrial chromosome name. This should be consistent with the chromosome names in the bam file. |
| has_UMI | whether the protocol contains UMI (default: TRUE) |
| UMI_cor | correct UMI sequencing error: 0 means no correction, 1 means simple correction and merge UMI with distance 1. 2 means merge on both UMI alignment position match. |
| gene_fl | whether to remove low abundance genes. A gene is considered to have low abundance if only one copy of one UMI is associated with it. |
| keep_mapped_bam | TRUE if feature mapped bam file should be retained. |
| nthreads | number of threads to use. (default: 1) |

Value

no return

Examples

```
## Not run:
sc_count_aligned_bam(
  inbam = "aligned.bam",
  outbam = "mapped.bam",
  annofn = c("MusMusculus-GRCm38p4-UCSC.gff3", "ERCC92_anno.gff3"),
  outdir = "output",
  bc_anno = "barcodes.csv"
)

## End(Not run)
```

| | |
|----------------|-----------------------|
| sc_demultiplex | <i>sc_demultiplex</i> |
|----------------|-----------------------|

Description

Process bam file by cell barcode, output to outdir/count/[cell_id].csv. the output contains information for all reads that can be mapped to exons. including the gene id, UMI of that read and the distance to transcript end position.

Usage

```
sc_demultiplex(inbam, outdir, bc_anno, max_mis = 1, bam_tags = list(am
  = "YE", ge = "GE", bc = "BC", mb = "OX"), mito = "MT",
  has_UMI = TRUE, nthreads = 1)
```

Arguments

| | |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| inbam | input bam file. This should be the output of sc_exon_mapping |
| outdir | output folder |
| bc_anno | barcode annotation, first column is cell id, second column is cell barcode sequence |
| max_mis | maximum mismatch allowed in barcode. (default: 1) |
| bam_tags | list defining BAM tags where mapping information is stored. <ul style="list-style-type: none"> • "am": mapping status tag • "ge": gene id • "bc": cell barcode tag • "mb": molecular barcode tag |
| mito | mitochondrial chromosome name. This should be consistent with the chromosome names in the bam file. |
| has_UMI | whether the protocol contains UMI (default: TRUE) |
| nthreads | number of threads to use. (default: 1) |

Value

no return

Examples

```
data_dir="celseq2_demo"
barcode_annotation_fn = system.file("extdata", "barcode_anno.csv",
  package = "scPipe")
## Not run:
# refer to the vignettes for the complete workflow
...
sc_demultiplex(file.path(data_dir, "out.map.bam"),
  data_dir,
  barcode_annotation_fn,has_UMI=FALSE)
...

## End(Not run)
```

sc_demultiplex_and_count

sc_demultiplex_and_count

Description

Wrapper to run [sc_demultiplex](#) and [sc_gene_counting](#) with a single command

Usage

```
sc_demultiplex_and_count(inbam, outdir, bc_anno, max_mis = 1,
  bam_tags = list(am = "YE", ge = "GE", bc = "BC", mb = "OX"),
  mito = "MT", has_UMI = TRUE, UMI_cor = 1, gene_fl = FALSE,
  nthreads = 1)
```

Arguments

| | |
|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| inbam | input bam file. This should be the output of <code>sc_exon_mapping</code> |
| outdir | output folder |
| bc_anno | barcode annotation, first column is cell id, second column is cell barcode sequence |
| max_mis | maximum mismatch allowed in barcode. (default: 1) |
| bam_tags | list defining BAM tags where mapping information is stored. <ul style="list-style-type: none"> "am": mapping status tag "ge": gene id "bc": cell barcode tag "mb": molecular barcode tag |
| mito | mitochondrial chromosome name. This should be consistent with the chromosome names in the bam file. |
| has_UMI | whether the protocol contains UMI (default: TRUE) |

| | |
|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| UMI_cor | correct UMI sequencing error: 0 means no correction, 1 means simple correction and merge UMI with distance 1. 2 means merge on both UMI alignment position match. |
| gene_fl | whether to remove low abundance genes. A gene is considered to have low abundance if only one copy of one UMI is associated with it. |
| nthreads | number of threads to use. (default: 1) |

Value

no return

Examples

```
## Not run:
refer to the vignettes for the complete workflow, replace demultiplex and
count with single command:
...
sc_demultiplex_and_count(
  file.path(data_dir, "out.map.bam"),
  data_dir,
  barcode_annotation_fn,
  has_UMI = FALSE
)
...
## End(Not run)
```

sc_detect_bc

sc_detect_bc

Description

Detect cell barcode and generate the barcode annotation

Usage

```
sc_detect_bc(infq, outcsv, prefix = "CELL_", bc_len, max_reads = 1e+06,
  min_count = 10, number_of_cells = 10000, max_mismatch = 1,
  white_list_file = NULL)
```

Arguments

| | |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------|
| infq | input fastq file, should be the output file of sc_trim_barcode |
| outcsv | output barcode annotation |
| prefix | the prefix of cell name (default: 'CELL_') |
| bc_len | the length of cell barcode, should be consistent with b11+b12 in sc_trim_barcode |
| max_reads | the maximum of reads processed (default: 1,000,000) |
| min_count | minimum counts to keep, barcode will be discarded if it has lower count. Default value is 10. This should be set according to max_reads. |

| | |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| number_of_cells | number of cells kept in result. (default: 10000) |
| max_mismatch | the maximum mismatch allowed. Barcodes within this number will be considered as sequence error and merged. (default: 1) |
| white_list_file | a file that list all the possible barcodes each row is a barcode sequence. the list for 10x can be found at: https://community.10xgenomics.com/t5/Data-Sharing/List-of-valid-cellular-barcodes/td-p/527 (default: NULL) |

Value

no return

Examples

```
## Not run:
# `sc_detect_bc`` should run before `sc_demultiplex` for
# Drop-seq or 10X protocols
sc_detect_bc("input.fastq", "output.cell_index.csv", bc_len=8)
sc_demultiplex(..., "output.cell_index.csv")

## End(Not run)
```

| | |
|-----------------|------------------------|
| sc_exon_mapping | <i>sc_exon_mapping</i> |
|-----------------|------------------------|

Description

Map aligned reads to exon annotation. The result will be written into optional fields in bam file with different tags. Following this link for more information regarding to bam file format: <http://samtools.github.io/hts-specs>

The function can accept multiple bam file as input, if multiple bam file is provided and the 'bc_len' is zero, then the function will use the barcode in the 'barcode_vector' to insert into the 'bc' bam tag. So the length of 'barcode_vector' and the length of 'inbam' should be the same. If this is the case then the 'max_mis' argument in 'sc_demultiplex' should be zero. If 'bc_len' is larger than zero, then the function will still seek for barcode in fastq headers with given length. In this case each bam file is not treated as from a single cell.

Usage

```
sc_exon_mapping(inbam, outbam, annofn, bam_tags = list(am = "YE", ge =
  "GE", bc = "BC", mb = "OX"), bc_len = 8, barcode_vector = "",
  UMI_len = 6, stnd = TRUE, fix_chr = FALSE, nthreads = 1)
```

Arguments

| | |
|--------|----------------------------------------------------------|
| inbam | input aligned bam file. can have multiple files as input |
| outbam | output bam filename |

| | |
|----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| annofn | single string or vector of gff3 annotation filenames, data.frame in SAF format or GRanges object containing complete gene_id metadata column. |
| bam_tags | list defining BAM tags where mapping information is stored. <ul style="list-style-type: none"> • "am": mapping status tag • "ge": gene id • "bc": cell barcode tag • "mb": molecular barcode tag |
| bc_len | total barcode length |
| barcode_vector | a list of barcode if each individual bam is a single cell. (default: NULL). The barcode should be of the same length for each cell. |
| UMI_len | UMI length |
| stnd | TRUE to perform strand specific mapping. (default: TRUE) |
| fix_chr | TRUE to add 'chr' to chromosome names, MT to chrM. (default: FALSE) |
| nthreads | number of threads to use. (default: 1) |

Value

generates a bam file with exons assigned

Examples

```

data_dir="celseq2_demo"
ERCCanno_fn = system.file("extdata", "ERCC92_anno.gff3",
  package = "scPipe")
## Not run:
# for the complete workflow, refer to the vignettes
...
sc_exon_mapping(file.path(data_dir, "out.aln.bam"),
  file.path(data_dir, "out.map.bam"),
  ERCCanno_fn)
...
## End(Not run)

```

sc_gene_counting *sc_gene_counting*

Description

Generate gene counts matrix with UMI deduplication

Usage

```
sc_gene_counting(outdir, bc_anno, UMI_cor = 2, gene_fl = FALSE)
```

Arguments

| | |
|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| outdir | output folder containing sc_demultiplex output |
| bc_anno | barcode annotation comma-separated-values, first column is cell id, second column is cell barcode sequence |
| UMI_cor | correct UMI sequencing error: 0 means no correction, 1 means simple correction and merge UMI with distance 1. 2 means merge on both UMI alignment position match. |
| gene_fl | whether to remove low abundance genes. A gene is considered to have low abundance if only one copy of one UMI is associated with it. |

Value

no return

Examples

```
data_dir="celseq2_demo"
barcode_annotation_fn = system.file("extdata", "barcode_anno.csv",
package = "scPipe")
## Not run:
# refer to the vignettes for the complete workflow
...
sc_gene_counting(data_dir, barcode_annotation_fn)
...
## End(Not run)
```

| | |
|----------------|--------------------------------------------------------------------------------------|
| sc_sample_data | <i>a small sample scRNA-seq counts dataset to demonstrate capabilities of scPipe</i> |
|----------------|--------------------------------------------------------------------------------------|

Description

This data set contains counts for high variable genes for 100 cells. The cells have different cell types. The data contains raw read counts. The cells are chosen randomly from 384 cells and they did not go through quality controls. The rows names are Ensembl gene ids and the columns are cell names, which is the well position in the 384 plates.

Usage

```
sc_sample_data
```

Format

a matrix instance, one row per gene.

Value

NULL, but makes a matrix of count data

Author(s)

Luyi Tian

Source

Christin Biben (WEHI). She FACS sorted cells from several immune cell types including B cells, granulocyte and some early progenitors.

Examples

```
# use the example dataset to perform quality control
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication
sce = detect_outlier(sce)

plot_QC_pairs(sce)
```

sc_sample_qc

quality control information for a small sample scRNA-seq dataset to demonstrate capabilities of scPipe.

Description

This data.frame contains cell quality control information for the 100 cells. For each cell it has:

- unaligned the number of unaligned reads.
- aligned_unmapped the number of reads that aligned to genome but fail to map to any features.
- mapped_to_exon is the number of reads that mapped to exon.
- mapped_to_intron is the number of reads that mapped to intron.
- ambiguous_mapping is the number of reads that mapped to multiple features. They are not considered in the following analysis.
- mapped_to_ERCC is the number of reads that mapped to ERCC spike-in controls.
- mapped_to_MT is the number of reads that mapped to mitochondrial genes.
- total_count_per_cell is the number of reads that mapped to exon after UMI deduplication. In contrast, 'mapped_to_exon' is the number of reads mapped to exon before UMI deduplication.
- number_of_genes is the number of genes detected for each cells
- non_ERCC_percent is 1 - (percentage of ERCC reads). Reads are UMI deduplicated.
- non_mt_percent is 1 - (percentage of mitochondrial reads). Reads are UMI deduplicated.
- non_ribo_percent is 1 - (percentage of ribosomal reads). Reads are UMI deduplicated.

Usage

```
sc_sample_qc
```

Format

a data.frame instance, one row per cell.

Value

NULL, but makes a data frame with cell quality control data.frame

Author(s)

Luyi Tian

Source

Christin Biben (WEHI). She FACS sorted cells from several immune cell types including B cells, granulocyte and some early progenitors.

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
head(QC_metrics(sce))
plot_mapping(sce, percentage=TRUE, dataname="sc_sample")
```

sc_trim_barcode

sc_trim_barcode

Description

Reformat fastq files so barcode and UMI sequences are moved from the sequence into the read name.

Usage

```
sc_trim_barcode(outfq, r1, r2 = NULL, read_structure = list(bs1 = -1,
  b11 = 0, bs2 = 6, b12 = 8, us = 0, ul = 6),
  filter_settings = list(rmlow = TRUE, rmN = TRUE, minq = 20, numbq = 2))
```

Arguments

| | |
|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| outfq | the output fastq file, which reformat the barcode and UMI into the read name. Files ending in .gz will be automatically compressed. |
| r1 | read one for pair-end reads. This read should contain the transcript. |
| r2 | read two for pair-end reads, NULL if single read. (default: NULL) |
| read_structure | a list containing the read structure configuration: <ul style="list-style-type: none"> bs1: starting position of barcode in read one. -1 if no barcode in read one. |

- b11: length of barcode in read one, if there is no barcode in read one this number is used for trimming beginning of read one.
- bs2: starting position of barcode in read two
- b12: length of barcode in read two
- us: starting position of UMI
- ul: length of UMI

filter_settings

A list contains read filter settings:

- rmlow whether to remove the low quality reads.
- rmN whether to remove reads that contains N in UMI or cell barcode.
- minq the minimum base pair quality that we allowed
- numbq the maximum number of base pair that have quality below numbq

Details

Positions used in this function are 0-indexed, so they start from 0 rather than 1. The default read structure in this function represents CEL-seq paired-ended reads. This contains a transcript in the first read, a UMI in the first 8bp of the second read followed by a 6bp barcode. So the read structure will be : `list(bs1=-1, b11=0, bs2=6, b12=8, us=0, ul=6)`. `bs1=-1, b11=0` indicates negative start position and zero length for the barcode on read one, this is used to denote "no barcode" on read one. `bs2=6, b12=8` indicates there is a barcode in read two that starts at the 7th base with length 8bp. `us=0, ul=6` indicates a UMI from first base of read two and the length in 6bp.

For a typical Drop-seq experiment the read structure will be `list(bs1=-1, b11=0, bs2=0, b12=12, us=12, ul=8)`, which means the read one only contains transcript, the first 12bp in read two are index, followed by a 8bp UMI.

Value

generates a trimmed fastq file named outfq

Examples

```
data_dir="celseq2_demo"
## Not run:
# for the complete workflow, refer to the vignettes
...
sc_trim_barcode(file.path(data_dir, "combined.fastq"),
  file.path(data_dir, "simu_R1.fastq"),
  file.path(data_dir, "simu_R2.fastq"))
...

## End(Not run)
```

UMI_duplication

UMI duplication statistics for a small sample scRNA-seq dataset to demonstrate capabilities of scPipe

Description

This data.frame contains UMI duplication statistics, where the first column is the number of duplication, and the second column is the count of UMIs.

Usage

```
sc_sample_qc
```

Format

a data.frame instance, one row per cell.

Value

NULL, but makes a data frame with UMI duplication statistics

Author(s)

Luyi Tian

Source

Christin Biben (WEHI). She FACS sorted cells from several immune cell types including B cells, granulocyte and some early progenitors.

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

head(UMI_dup_info(sce))
```

UMI_dup_info

Get or set UMI duplication results in a SingleCellExperiment object

Description

Get or set UMI duplication results in a SingleCellExperiment object

Usage

```
UMI_dup_info(object)

UMI_dup_info(object) <- value

UMI_dup_info.sce(object)

## S4 method for signature 'SingleCellExperiment'
UMI_dup_info(object)

## S4 replacement method for signature 'SingleCellExperiment'
UMI_dup_info(object) <- value
```


Arguments

object A [SingleCellExperiment](#) object.
value Value to be assigned to corresponding object.

Value

a dataframe of cell UMI duplication information
A DataFrame of UMI duplication results.

Author(s)

Luyi Tian

Examples

```
data("sc_sample_data")
data("sc_sample_qc")
sce = SingleCellExperiment(assays = list(counts = as.matrix(sc_sample_data)))
organism(sce) = "mmusculus_gene_ensembl"
gene_id_type(sce) = "ensembl_gene_id"
QC_metrics(sce) = sc_sample_qc
demultiplex_info(sce) = cell_barcode_matching
UMI_dup_info(sce) = UMI_duplication

head(UMI_dup_info(sce))
```

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