Package 'scPipe'

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Title pipeline for single cell RNA-seq data analysis

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Type Package

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biocViews Software, Sequencing, RNASeq, GeneExpression, SingleCell, Visualization, SequenceMatching, Preprocessing, QualityControl, GenomeAnnotation

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, zlibbioc

Imports Rhtslib, biomaRt, GGally, MASS, mclust, Rcpp (>= 0.11.3), reshape, BiocGenerics, robustbase, scales, utils, stats, S4Vectors, SummarizedExperiment

License GPL (>= 2)

Encoding UTF-8

RoxygenNote 6.0.1

NeedsCompilation yes

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

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

Suggests knitr, rmarkdown, testthat

VignetteBuilder knitr

R topics documented:

`calculate_QC_metrics` *Calcuate QC metrics from gene count matrix*

Description

Calcuate 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 retrived 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 retrived 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 `listAttributes` from `biomaRt` package. The commonly used id types are 'external_gene_name', 'ensembl_gene_id' or 'entrezgene'.
all	logic. For genes that cannot covert 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` *create_report*

Description

create an HTML report using data generated by proprocessing step.

Usage

```
create_report(sample_name, outdir, r1 = "NA", r2 = "NA", outfq = "NA",
  read_structure = list(bs1 = 0, bl1 = 0, bs2 = 0, bl2 = 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
`stnd`	whether to perform strand specific mapping
`fix_chr`	add 'chr' to chromosome names, fix inconsistant 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, bl1=2, bs2=6, bl2=8, us=0, ul=6),
       filter_settings=list(rmlow=TRUE, rmN=TRUE, minq=20, numbq=2),
       align_bam="align.bam",
       genome_index="mouse.index",
       map_bam="aligned.mapped.bam",
       exon_anno="exon_anno.gff3",
       stnd=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 *create a SingleCellExperiment object from data folder generated by preprocessing step*

Description

after we run `sc_gene_counting` and finish the preprocessing step. `create_sce_by_dir` can be used to generate the SingleCellExperiment obeject 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

`datadir`	the directory that contains all the data and ‘stat‘ subfolder.
`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‘
pheno_data	the external phenotype data that linked to each single cell. This should be an AnnotatedDataFrame object
report	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 obeject from the folder that contains gene count matrix and QC statistics.

Value

a SingleCellExperiment object

Examples

```
## Not run:
# the sce can be created fron 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 demultiplx 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 demultiplx 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_genes_by_GO *Get genes related to certain GO terms from biomart database*

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 `listAttributes` from `biomaRt` 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 `listDatasets` from `biomaRt` 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'))
```

---

organism.sce *Get or set* `organism` *from a SingleCellExperiment object*

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 *plot_demultiplex*

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 *Plot mapping statistics for* SingleCellExperiment *object.*

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 *Plot GGAlly pairs plot of QC statistics from* SingleCellExperiment *object*

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 *Plot UMI duplication frequency*

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

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

Value

a dataframe of quality control matrics

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 *Remove outliers in* SingleCellExperiment

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 *scPipe - single cell RNA-seq pipeline*

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_demultiplex *sc_demultiplex*

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)
```

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. • "am": mapping status tag • "ge": gene id • "bc": cell barcode tag • "mb": molecular barcode tag
mito	mitochondrial chromosome name. This should be consistant with the chromosome names in the bam file.
has_UMI	whether the protocol contains UMI (default: TRUE)

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)
```

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. • "am": mapping status tag • "ge": gene id • "bc": cell barcode tag • "mb": molecular barcode tag
mito	mitochondrial chromosome name. This should be consistant 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.
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

```
## 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, suffix = "CELL_", bc_len, max_reads = 1e+06,
  min_count = 10, max_mismatch = 1)
```

Arguments

infq	input fastq file, shoule be the output file of sc_trim_barcode
outcsv	output barcode annotation
suffix	the suffix of cell name (default: 'CELL_')
bc_len	the length of cell barcode, should be consistent with bl1+bl2 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.
max_mismatch	the maximum mismatch allowed. Barcodes within this number will be considered as sequence error and merged. (default: 1)

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 *sc_exon_mapping*

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

Usage

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

Arguments

inbam	input aligned bam file
outbam	output bam filename
annofn	single string or vector of gff3 annotation filenames
bam_tags	list defining BAM tags where mapping information is stored. • "am": mapping status tag • "ge": gene id • "bc": cell barcode tag • "mb": molecular barcode tag
bc_len	total barcode length
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)

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 = 1, 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.
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 *a small sample scRNA-seq counts dataset to demonstrate capabilities of scPipe*

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 wall 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, bl1 =
  0, bs2 = 6, bl2 = 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.
`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: • bs1: starting position of barcode in read one. -1 if no barcode in read one. • bl1: 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 • bl2: 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, bl1=0, bs2=6, bl2=8, us=0, ul=6)`. `bs1=-1, bl1=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, bl2=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, bl1=0, bs2=0, bl2=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 dulication 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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