

# Package ‘scPipe’

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**Title** Pipeline for single cell multi-omic data pre-processing

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**Type** Package

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

**Description** A preprocessing pipeline for single cell RNA-seq/ATAC-seq data that starts from the fastq files and produces a feature 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 (>= 4.2.0), SingleCellExperiment

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

**Imports** AnnotationDbi, basilisk, BiocGenerics, biomaRt, Biostrings, data.table, dplyr, DropletUtils, flexmix, GenomicRanges, GenomicAlignments, GGally, ggplot2, glue (>= 1.3.0), grDevices, graphics, hash, IRanges, magrittr, MASS, Matrix (>= 1.5.0), mclust, methods, MultiAssayExperiment, org.Hs.eg.db, org.Mm.eg.db, purrr, Rcpp (>= 0.11.3), reshape, reticulate, Rhtslib, rlang, robustbase, Rsamtools, Rsubread, rtracklayer, SummarizedExperiment, S4Vectors, scales, stats, stringr, tibble, tidyr, tools, utils, vctrs (>= 0.5.2)

**SystemRequirements** C++11, GNU make

**License** GPL (>= 2)

**Encoding** UTF-8

**RoxygenNote** 7.2.3

**NeedsCompilation** yes

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

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

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knitr, locStra, plotly, rmarkdown, RColorBrewer, readr,  
reshape2, RANN, shiny, scater (>= 1.11.0), testthat, xml2, umap

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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

<code>x</code>	a vector of mahalanobis distance
<code>df</code>	degree of freedom for chi-square distribution
<code>conf</code>	confidence for linear regression

### Value

cell names of outliers

---

`anno_import`     *Import gene annotation*

---

### 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](http://www.github.com/LuyiTian/scPipe) with details.

### Usage

```
anno_import(filename)
```

### Arguments

<code>filename</code>	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

```
## Not run:
anno <- system.file("extdata", "ensembl_hg38_chrY.gtf.gz", package = "scPipe")
saf_chrY <- anno_to_saf(rtracklayer::import(anno))

## End(Not run)
```

---

calculate\_QC\_metrics *Calculate QC metrics from gene count matrix*

---

## 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

## 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)

```

---

```
check_barcode_start_position
```

*Check Valid Barcode Start Position*

---

**Description**

Checks to see if the given barcode start position (bstart) is valid for the fastq file. If the found barcode percentage is less than the given threshold, a new barcode start position is searched for by checking every position from the start of each read to 10 bases after the bstart

**Usage**

```

check_barcode_start_position(
  fastq,
  barcode_file,
  barcode_file_realname,
  bstart,
  blength,
  search_lines,
  threshold
)

```

**Arguments**

fastq	file containing reads
barcode_file	csv file
barcode_file_realname	the real name of the csv file
bstart	the start position for barcodes in the given reads
blength	length of each barcode
search_lines	the number of fastq lines to use for the check
threshold	the minimum percentage of found barcodes to accept for the program to continue



**Value**

Boolean; TRUE if program can continue execution, FALSE otherwise.

---

convert_geneid	<i>convert the gene ids of a SingleCellExperiment object</i>
----------------	--

---

**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

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

## Value

file path of the created compiled document.

## 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, 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 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, b11=2, bs2=6, b12=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",
              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	<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	<i>demultiplex_info</i>
------------------	-------------------------

---

**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 <code>SingleCellExperiment</code> 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)
```

---

feature\_info

*Get or set feature\_info from a SingleCellExperiment object*


---

**Description**

Get or set feature\_info from a SingleCellExperiment object



**Usage**

```
feature_info(object)

feature_info(object) <- value

feature_info.sce(object)

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

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

**Arguments**

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

**Value**

a dataframe of feature info for scATAC-seq data  
A DataFrame of feature information

**Author(s)**

Shani Amarasinghe

---

feature_type	<i>Get or set feature_type from a SingleCellExperiment object</i>
--------------	---

---

**Description**

Get or set feature\_type from a SingleCellExperiment object

**Usage**

```
feature_type(object)

feature_type(object) <- value

feature_type.sce(object)

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

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

**Arguments**

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

**Value**

the feature type used in feature counting for scATAC-Seq data  
 A string representing the feature type

**Author(s)**

Shani Amarasinghe

---

gene_id_type	<i>Get or set gene_id_type from a SingleCellExperiment object</i>
--------------	---

---

**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_chromosomes	<i>Get Chromosomes</i>
-----------------	------------------------

---

**Description**

Gets a list of NamedList of chromosomes and the reference length acquired through the bam index file.

**Usage**

```
get_chromosomes(bam, keep_contigs = "^chr")
```

**Arguments**

bam                    file path to the bam file to get data from  
keep\_contigs        regular expression used with grepl to filter reference names

**Value**

a named list where element names are chromosomes reference names and elements are integer lengths

---

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()
```

---

<code>get_genes_by_GO</code>	<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	<i>Get read structure for particular scRNA-seq protocol</i>
--------------	---

---

### 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](http://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        *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	<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**

object                A [SingleCellExperiment](#) object.  
 value                 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))
```

---

read\_cells

*Read Cell barcode file*

---

**Description**

Read Cell barcode file

**Usage**

```
read_cells(cells)
```

**Arguments**

cells            the file path to the barcode file. Assumes one barcode per line or barcode csv.  
Or, cells can be a comma delimited string of barcodes

**Value**

a character vector of the provided barcodes

---

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\_aligning

*aligning the demultiplexed FASTQ reads using the Rsubread:align()*


---

## Description

after we run the `sc_trim_barcode` or `sc_atac_trim_barcode` to demultiplex the fastq files, we are using this function to align those fastq files to a known reference.

## Usage

```
sc_aligning(
  R1,
  R2 = NULL,
  tech = "atac",
  index_path = NULL,
  ref = NULL,
  output_folder = NULL,
  output_file = NULL,
  input_format = "FASTQ",
  output_format = "BAM",
  type = "dna",
  nthreads = 1
)
```

## Arguments

R1	a mandatory character vector including names of files that include sequence reads to be aligned. For paired-end reads, this gives the list of files including first reads in each library. File format is FASTQ/FASTA by default.
R2	a character vector, the second fastq file, which is required if the data is paired-end
tech	a character string giving the sequencing technology. Possible value includes "atac" or "rna"
index_path	character string specifying the path/basename of the index files, if the Rsubread genome build is available
ref	a character string specifying the path to reference genome file (.fasta, .fa format)
output_folder	a character string, the name of the output folder
output_file	a character vector specifying names of output files. By default, names of output files are set as the file names provided in R1 added with an suffix string
input_format	a string indicating the input format
output_format	a string indicating the output format
type	type of sequencing data ('RNA' or 'DNA')
nthreads	numeric value giving the number of threads used for mapping.

**Value**

the file path of the output aligned BAM file

**Examples**

```
## Not run:  
sc_aligning(index_path,  
            tech = 'atac',  
            R1,  
            R2,  
            nthreads = 6)  
  
## End(Not run)
```

---

sc\_atac\_bam\_tagging    *BAM tagging*

---

**Description**

Demultiplexes the reads

**Usage**

```
sc_atac_bam_tagging(  
  inbam,  
  output_folder = NULL,  
  bc_length = NULL,  
  bam_tags = list(bc = "CB", mb = "OX"),  
  nthreads = 1  
)
```

**Arguments**

inbam	The input BAM file
output_folder	The path of the output folder
bc_length	The length of the cellular barcodes
bam_tags	The BAM tags
nthreads	The number of threads

**Details**

```
sc_atac_bam_tagging()
```

**Value**

file path of the resultant demultiplexed BAM file.

**Examples**

```

r1 <- system.file("extdata", "small_chr21_R1.fastq.gz", package="scPipe")
r2 <- system.file("extdata", "small_chr21_R3.fastq.gz", package="scPipe")
barcode_fastq <- system.file("extdata", "small_chr21_R2.fastq.gz", package="scPipe")
out <- tempdir()

sc_atac_trim_barcode(r1=r1, r2=r2, bc_file=barcode_fastq, output_folder=out)

demux_r1 <- file.path(out, "demux_completematch_small_chr21_R1.fastq.gz")
demux_r2 <- file.path(out, "demux_completematch_small_chr21_R3.fastq.gz")
reference <- system.file("extdata", "small_chr21.fa", package="scPipe")

aligned_bam <- sc_aligning(ref=reference, R1=demux_r1, R2=demux_r2, nthreads=6, output_folder=out)

out_bam <- sc_atac_bam_tagging(
  inbam = aligned_bam,
  output_folder = out,
  nthreads = 6)

```

---

sc\_atac\_cell\_calling *identifying true vs empty cells*

---

**Description**

the methods to call true cells are of various ways. implement (i.e. filtering from scATAC-Pro as default

**Usage**

```

sc_atac_cell_calling(
  mat,
  cell_calling = "filter",
  output_folder,
  genome_size = NULL,
  cell_qc_metrics_file = NULL,
  lower = NULL,
  min_uniq_frags = 3000,
  max_uniq_frags = 50000,
  min_frac_peak = 0.3,
  min_frac_tss = 0,
  min_frac_enhancer = 0,
  min_frac_promoter = 0.1,
  max_frac_mito = 0.15
)

```

**Arguments**

mat	the feature by cell matrix.
cell_calling	the cell calling approach, possible options were "emptydrops" , "cellranger" and "filter". But we opten to using "filter" as it was most robust. "emptydrops" is still an opition for data with large umber of cells.
output_folder	output directory for the cell called matrix.
genome_size	genome size for the data in feature by cell matrix.
cell_qc_metrics_file	quality per barcode file for the barcodes in the matrix if using the cellranger or filter options.
lower	the lower threshold for the data if using the emptydrops function for cell calling.
min_uniq_frags	The minimum number of required unique fragments required for a cell (used for filter cell calling)
max_uniq_frags	The maximum number of required unique fragments required for a cell (used for filter cell calling)
min_frac_peak	The minimum proportion of fragments in a cell to overlap with a peak (used for filter cell calling)
min_frac_tss	The minimum proportion of fragments in a cell to overlap with a tss (used for filter cell calling)
min_frac_enhancer	The minimum proportion of fragments in a cell to overlap with a enhancer sequence (used for filter cell calling)
min_frac_promoter	The minimum proportion of fragments in a cell to overlap with a promoter sequence (used for filter cell calling)
max_frac_mito	The maximum proportion of fragments in a cell that are mitochondrial (used for filter cell calling)

**Examples**

```
## Not run:
sc_atac_cell_calling <- function(mat,
  cell_calling,
  output_folder,
  genome_size = NULL,
  cell_qc_metrics_file = NULL,
  lower = NULL)

## End(Not run)
```

---

`sc_atac_create_cell_qc_metrics`*generating a file useful for producing the qc plots*

---

**Description**

uses the peak file and annotation files for features

**Usage**

```
sc_atac_create_cell_qc_metrics(  
  frags_file,  
  peaks_file,  
  promoters_file,  
  tss_file,  
  enhs_file,  
  output_folder  
)
```

**Arguments**

<code>frags_file</code>	The fragment file
<code>peaks_file</code>	The peak file
<code>promoters_file</code>	The path of the promoter annotation file
<code>tss_file</code>	The path of the tss annotation file
<code>enhs_file</code>	The path of the enhs annotation file
<code>output_folder</code>	The path of the output folder for resultant files

**Value**

Nothing (Invisible 'NULL')

---

`sc_atac_create_fragments`*Generating the popular fragments for scATAC-Seq data*

---

**Description**

Takes in a tagged and sorted BAM file and outputs the associated fragments in a .bed file



**Usage**

```

sc_atac_create_fragments(
    inbam,
    output_folder = "",
    min_mapq = 30,
    nproc = 1,
    cellbarcode = "CB",
    chromosomes = "^chr",
    readname_barcode = NULL,
    cells = NULL,
    max_distance = 5000,
    min_distance = 10,
    chunksize = 5e+05
)

```

**Arguments**

inbam	The tagged, sorted and duplicate-free input BAM file
output_folder	The path of the output folder
min_mapq	: int Minimum MAPQ to retain fragment
nproc	: int, optional Number of processors to use. Default is 1.
cellbarcode	: str Tag used for cell barcode. Default is CB (used by cellranger)
chromosomes	: str, optional Regular expression used to match chromosome names to include in the output file. Default is "(?i)^chr" (starts with "chr", case-insensitive). If None, use all chromosomes in the BAM file.
readname_barcode	: str, optional Regular expression used to match cell barcode stored in read name. If None (default), use read tags instead. Use "[^:]*" to match all characters before the first colon (":").
cells	: str File containing list of cell barcodes to retain. If None (default), use all cell barcodes found in the BAM file.
max_distance	: int, optional Maximum distance between integration sites for the fragment to be retained. Allows filtering of implausible fragments that likely result from incorrect mapping positions. Default is 5000 bp.
min_distance	: int, optional Minimum distance between integration sites for the fragment to be retained. Allows filtering implausible fragments that likely result from incorrect mapping positions. Default is 10 bp.
chunksize	: int Number of BAM entries to read through before collapsing and writing fragments to disk. Higher chunksize will use more memory but will be faster.

**Value**

returns NULL

---

sc\_atac\_create\_report *HTML report generation*

---

### Description

Generates a HTML report using the output folder produced by the pipeline

### Usage

```
sc_atac_create_report(  
  input_folder,  
  output_folder = NULL,  
  organism = NULL,  
  sample_name = NULL,  
  feature_type = NULL,  
  n_barcode_subset = 500  
)
```

### Arguments

input_folder	The path of the folder produced by the pipeline
output_folder	The path of the output folder to store the HTML report in
organism	A string indicating the name of the organism being analysed
sample_name	A string indicating the name of the sample
feature_type	A string indicating the type of the feature ('genome_bin' or 'peak')
n_barcode_subset	if you require only to visualise stats for a sample of barcodes to improve processing time (integer)

### Value

the path of the output file

---

sc\_atac\_create\_sce *sc\_atac\_create\_sce()*

---

### Description

sc\_atac\_create\_sce()

**Usage**

```
sc_atac_create_sce(
  input_folder = NULL,
  organism = NULL,
  sample_name = NULL,
  feature_type = NULL,
  pheno_data = NULL,
  report = FALSE
)
```

**Arguments**

input_folder	The output folder produced by the pipeline
organism	The type of the organism
sample_name	The name of the sample
feature_type	The type of the feature
pheno_data	The pheno data
report	Whether or not a HTML report should be produced

**Value**

a SingleCellExperiment object created from the scATAC-Seq data provided

**Examples**

```
## Not run:
sc_atac_create_sce(
  input_folder = input_folder,
  organism = "hg38",
  feature_type = "peak",
  report = TRUE)

## End(Not run)
```

---

sc\_atac\_emptydrops\_cell\_calling  
*empty drops cell calling*

---

**Description**

The empty drops cell calling method

**Usage**

```
sc_atac_emptydrops_cell_calling(mat, output_folder, lower = NULL)
```

**Arguments**

mat	The input matrix
output_folder	The path of the output folder
lower	The lower threshold for the data if using the emptydrops function for cell calling.

---

sc\_atac\_feature\_counting

*generating the feature by cell matrix*

---

**Description**

feature matrix is created using a given demultiplexed BAM file and a selected feature type

**Usage**

```
sc_atac_feature_counting(
  fragment_file,
  feature_input = NULL,
  bam_tags = list(bc = "CB", mb = "OX"),
  feature_type = "peak",
  organism = "hg38",
  cell_calling = "filter",
  sample_name = "",
  genome_size = NULL,
  promoters_file = NULL,
  tss_file = NULL,
  enhs_file = NULL,
  gene_anno_file = NULL,
  pheno_data = NULL,
  bin_size = NULL,
  yieldsize = 1e+06,
  n_filter_cell_counts = 200,
  n_filter_feature_counts = 10,
  exclude_regions = FALSE,
  excluded_regions_filename = NULL,
  output_folder = NULL,
  fix_chr = "none",
  lower = NULL,
  min_uniq_frags = 3000,
  max_uniq_frags = 50000,
  min_frac_peak = 0.3,
  min_frac_tss = 0,
  min_frac_enhancer = 0,
  min_frac_promoter = 0.1,
  max_frac_mito = 0.15,
```

```

    create_report = FALSE
)

```

### Arguments

fragment_file	The fragment file
feature_input	The feature input data e.g. the .narrowPeak file for peaks of a bed file format
bam_tags	The BAM tags
feature_type	The type of feature
organism	The organism type (contains hg19, hg38, mm10)
cell_calling	The desired cell calling method; either cellranger, emptydrops or filter.
sample_name	The sample name to identify which is the data is analysed for.
genome_size	The size of the genome (used for the cellranger cell calling method)
promoters_file	The path of the promoter annotation file (if the specified organism isn't recognised).
tss_file	The path of the tss annotation file (if the specified organism isn't recognised).
enhs_file	The path of the enhs annotation file (if the specified organism isn't recognised).
gene_anno_file	The path of the gene annotation file (gtf or gff3 format).
pheno_data	The phenotypic data as a data frame
bin_size	The size of the bins
yieldsize	The yield size
n_filter_cell_counts	An integer value to filter the feature matrix on the number of reads per cell (default = 200)
n_filter_feature_counts	An integer value to filter the feature matrix on the number of reads per feature (default = 10).
exclude_regions	Whether or not the regions (specified in the file) should be excluded
excluded_regions_filename	The filename of the file containing the regions to be excluded
output_folder	The output folder
fix_chr	Whether chr should be fixed or not
lower	the lower threshold for the data if using the emptydrops function for cell calling
min_uniq_frags	The minimum number of required unique fragments required for a cell (used for filter cell calling)
max_uniq_frags	The maximum number of required unique fragments required for a cell (used for filter cell calling)
min_frac_peak	The minimum proportion of fragments in a cell to overlap with a peak (used for filter cell calling)
min_frac_tss	The minimum proportion of fragments in a cell to overlap with a tss (used for filter cell calling)

min_frac_enhancer	The minimum proportion of fragments in a cell to overlap with an enhancer sequence (used for filter cell calling)
min_frac_promoter	The minimum proportion of fragments in a cell to overlap with a promoter sequence (used for filter cell calling)
max_frac_mito	The maximum proportion of fragments in a cell that are mitochondrial (used for filter cell calling)
create_report	Logical value to say whether to create the report or not (default = TRUE).

**Value**

None (invisible 'NULL')

**Examples**

```
## Not run:
sc_atac_feature_counting(
  fragment_file = fragment_file,
  cell_calling = "filter",
  exclude_regions = TRUE,
  feature_input = feature_file)

## End(Not run)
```

---

sc\_atac\_filter\_cell\_calling  
*filter cell calling*

---

**Description**

specify various qc cutoffs to select the desired cells

**Usage**

```
sc_atac_filter_cell_calling(
  mtx,
  cell_qc_metrics_file,
  min_uniq_frags = 0,
  max_uniq_frags = 50000,
  min_frac_peak = 0.05,
  min_frac_tss = 0,
  min_frac_enhancer = 0,
  min_frac_promoter = 0,
  max_frac_mito = 0.2
)
```

**Arguments**

mtx	The input matrix
cell_qc_metrics_file	A file containing qc statistics for each cell
min_uniq_frags	The minimum number of required unique fragments required for a cell
max_uniq_frags	The maximum number of required unique fragments required for a cell
min_frac_peak	The minimum proportion of fragments in a cell to overlap with a peak
min_frac_tss	The minimum proportion of fragments in a cell to overlap with a tss
min_frac_enhancer	The minimum proportion of fragments in a cell to overlap with a enhancer sequence
min_frac_promoter	The minimum proportion of fragments in a cell to overlap with a promoter sequence
max_frac_mito	The maximum proportion of fragments in a cell that are mitochondrial

---

sc\_atac\_peak\_calling    *sc\_atac\_peak\_calling()*

---

**Description**

sc\_atac\_peak\_calling()

**Usage**

```
sc_atac_peak_calling(
  inbam,
  ref = NULL,
  genome_size = NULL,
  output_folder = NULL
)
```

**Arguments**

inbam	The input tagged, sorted, duplicate-free input BAM file
ref	The reference genome file
genome_size	The size of the genome
output_folder	The path of the output folder

**Value**

None (invisible 'NULL')

## Examples

```
## Not run:
sc_atac_peak_calling(
  inbam,
  reference)

## End(Not run)
```

---

sc\_atac\_pipeline      *A convenient function for running the entire pipeline*

---

## Description

A convenient function for running the entire pipeline

## Usage

```
sc_atac_pipeline(
  r1,
  r2,
  bc_file,
  valid_barcode_file = "",
  id1_st = -0,
  id1_len = 16,
  id2_st = 0,
  id2_len = 16,
  rmN = TRUE,
  rmlow = TRUE,
  organism = NULL,
  reference = NULL,
  feature_type = NULL,
  remove_duplicates = FALSE,
  samtools_path = NULL,
  genome_size = NULL,
  bin_size = NULL,
  yieldsize = 1e+06,
  exclude_regions = TRUE,
  excluded_regions_filename = NULL,
  fix_chr = "none",
  lower = NULL,
  cell_calling = "filter",
  promoters_file = NULL,
  tss_file = NULL,
  enhs_file = NULL,
  gene_anno_file = NULL,
  min_uniq_frags = 3000,
  max_uniq_frags = 50000,
```



```

    min_frac_peak = 0.3,
    min_frac_tss = 0,
    min_frac_enhancer = 0,
    min_frac_promoter = 0.1,
    max_frac_mito = 0.15,
    report = TRUE,
    nthreads = 12,
    output_folder = NULL
)

```

## Arguments

r1	The first read fastq file
r2	The second read fastq file
bc_file	the barcode information, can be either in a fastq format (e.g. from 10x-ATAC) or from a .csv file (here the barcode is expected to be on the second column). Currently, for the fastq approach, this can be a list of barcode files.
valid_barcode_file	optional file path of the valid (expected) barcode sequences to be found in the bc_file (.txt, can be txt.gz). Must contain one barcode per line on the second column separated by a comma (default = ""). If given, each barcode from bc_file is matched against the barcode of best fit (allowing a hamming distance of 1). If a FASTQ bc_file is provided, barcodes with a higher mapping quality, as given by the fastq reads quality score are prioritised.
id1_st	barcode start position (0-indexed) for read 1, which is an extra parameter that is needed if the bc_file is in a .csv format.
id1_len	barcode length for read 1, which is an extra parameter that is needed if the bc_file is in a .csv format.
id2_st	barcode start position (0-indexed) for read 2, which is an extra parameter that is needed if the bc_file is in a .csv format.
id2_len	barcode length for read 2, which is an extra parameter that is needed if the bc_file is in a .csv format.
rmN	logical, whether to remove reads that contains N in UMI or cell barcode.
rmLow	logical, whether to remove reads that have low quality barcode sequences.
organism	The name of the organism e.g. hg38
reference	The reference genome file
feature_type	The feature type (either 'genome_bin' or 'peak')
remove_duplicates	Whether or not to remove duplicates (samtools is required)
samtools_path	A custom path of samtools to use for duplicate removal
genome_size	The size of the genome (used for the cellranger cell calling method)
bin_size	The size of the bins for feature counting with the 'genome_bin' feature type
yieldsize	The number of reads to read in for feature counting

exclude_regions	Whether or not the regions should be excluded
excluded_regions_filename	The filename of the file containing the regions to be excluded
fix_chr	Specify 'none', 'exclude_regions', 'feature' or 'both' to prepend the string "chr" to the start of the associated file
lower	the lower threshold for the data if using the emptydrops function for cell calling.
cell_calling	The desired cell calling method either cellranger, emptydrops or filter
promoters_file	The path of the promoter annotation file (if the specified organism isn't recognised)
tss_file	The path of the tss annotation file (if the specified organism isn't recognised)
enhs_file	The path of the enhs annotation file (if the specified organism isn't recognised)
gene_anno_file	The path of the gene annotation file (gtf or gff3 format)
min_uniq_frags	The minimum number of required unique fragments required for a cell (used for filter cell calling)
max_uniq_frags	The maximum number of required unique fragments required for a cell (used for filter cell calling)
min_frac_peak	The minimum proportion of fragments in a cell to overlap with a peak (used for filter cell calling)
min_frac_tss	The minimum proportion of fragments in a cell to overlap with a tss (used for filter cell calling)
min_frac_enhancer	The minimum proportion of fragments in a cell to overlap with an enhancer sequence (used for filter cell calling)
min_frac_promoter	The minimum proportion of fragments in a cell to overlap with a promoter sequence (used for filter cell calling)
max_frac_mito	The maximum proportion of fragments in a cell that are mitochondrial (used for filter cell calling)
report	Whether or not a HTML report should be produced
nthreads	The number of threads to use for alignment (sc_align) and demultiplexing (sc_atac_bam_tagging)
output_folder	The path of the output folder

**Value**

None (invisible 'NULL')

**Examples**

```
data.folder <- system.file("extdata", package = "scPipe", mustWork = TRUE)
r1 <- file.path(data.folder, "small_chr21_R1.fastq.gz")
r2 <- file.path(data.folder, "small_chr21_R3.fastq.gz")

# Using a barcode fastq file:
```

```
# barcodes in fastq format
barcode_fastq <- file.path(data.folder, "small_chr21_R2.fastq.gz")

## Not run:
sc_atac_pipeline(
  r1 = r1,
  r2 = r2,
  bc_file = barcode_fastq
)

## End(Not run)
```

---

sc\_atac\_pipeline\_quick\_test

*A function that tests the pipeline on a small test sample (without duplicate removal)*

---

### **Description**

A function that tests the pipeline on a small test sample (without duplicate removal)

### **Usage**

```
sc_atac_pipeline_quick_test()
```

### **Value**

None (invisible 'NULL')

---

sc\_atac\_plot\_cells\_per\_feature

*A histogram of the log-number of cells per feature*

---

### **Description**

A histogram of the log-number of cells per feature

### **Usage**

```
sc_atac_plot_cells_per_feature(sce)
```

### **Arguments**

sce            The SingleExperimentObject produced by the sc\_atac\_create\_sce function at the end of the pipeline

**Value**

returns NULL

---

sc\_atac\_plot\_features\_per\_cell

*A histogram of the log-number of features per cell*

---

**Description**

A histogram of the log-number of features per cell

**Usage**

sc\_atac\_plot\_features\_per\_cell(sce)

**Arguments**

sce	The SingleExperimentObject produced by the sc_atac_create_sce function at the end of the pipeline
-----	---

**Value**

returns NULL

---

sc\_atac\_plot\_features\_per\_cell\_ordered

*Plot showing the number of features per cell in ascending order*

---

**Description**

Plot showing the number of features per cell in ascending order

**Usage**

sc\_atac\_plot\_features\_per\_cell\_ordered(sce)

**Arguments**

sce	The SingleExperimentObject produced by the sc_atac_create_sce function at the end of the pipeline
-----	---

**Value**

returns NULL

---

sc\_atac\_plot\_fragments\_cells\_per\_feature

*A scatter plot of the log-number of fragments and log-number of cells per feature*

---

**Description**

A scatter plot of the log-number of fragments and log-number of cells per feature

**Usage**

```
sc_atac_plot_fragments_cells_per_feature(sce)
```

**Arguments**

sce	The SingleExperimentObject produced by the sc_atac_create_sce function at the end of the pipeline
-----	---

**Value**

returns NULL

---

sc\_atac\_plot\_fragments\_features\_per\_cell

*A scatter plot of the log-number of fragments and log-number of features per cell*

---

**Description**

A scatter plot of the log-number of fragments and log-number of features per cell

**Usage**

```
sc_atac_plot_fragments_features_per_cell(sce)
```

**Arguments**

sce	The SingleExperimentObject produced by the sc_atac_create_sce function at the end of the pipeline
-----	---

**Value**

returns NULL

---

sc\_atac\_plot\_fragments\_per\_cell

*A histogram of the log-number of fragments per cell*

---

**Description**

A histogram of the log-number of fragments per cell

**Usage**

```
sc_atac_plot_fragments_per_cell(sce)
```

**Arguments**

sce	The SingleExperimentObject produced by the sc_atac_create_sce function at the end of the pipeline
-----	---

**Value**

returns NULL

---

sc\_atac\_plot\_fragments\_per\_feature

*A histogram of the log-number of fragments per feature*

---

**Description**

A histogram of the log-number of fragments per feature

**Usage**

```
sc_atac_plot_fragments_per_feature(sce)
```

**Arguments**

sce	The SingleExperimentObject produced by the sc_atac_create_sce function at the end of the pipeline
-----	---

**Value**

returns NULL

---

 sc\_atac\_remove\_duplicates

*Removing PCR duplicates using samtools*


---

### Description

Takes in a BAM file and removes the PCR duplicates using the samtools markdup function. Requires samtools 1.10 or newer for statistics to be generated.

### Usage

```
sc_atac_remove_duplicates(inbam, samtools_path = NULL, output_folder = NULL)
```

### Arguments

inbam	The tagged, sorted and duplicate-free input BAM file
samtools_path	The path of the samtools executable (if a custom installation is to be specified)
output_folder	The path of the output folder

### Value

file path to a bam file created from samtools markdup

---

 sc\_atac\_tfidf

*generating the UMAPs for sc-ATAC-Seq preprocessed data*


---

### Description

Takes the binary matrix and generate a TF-IDF so the clustering can take place on the reduced dimensions.

### Usage

```
sc_atac_tfidf(binary.mat, output_folder = NULL)
```

### Arguments

binary.mat	The final, filtered feature matrix in binary format
output_folder	The path of the output folder

### Value

None (invisible 'NULL')

**Examples**

```
## Not run:
sc_atac_tfidf(binary.mat = final_binary_matrix)

## End(Not run)
```

---

sc\_atac\_trim\_barcode *demultiplex raw single-cell ATAC-Seq fastq reads*

---

**Description**

single-cell data need to be demultiplexed in order to retain the information of the cell barcodes the data belong to. Here we reformat fastq files so barcode/s (and if available the UMI sequences) are moved from the sequence into the read name. Since scATAC-Seq data are mostly paired-end, both ‘r1’ and ‘r2’ are demultiplexed in this function.

**Usage**

```
sc_atac_trim_barcode(
  r1,
  r2,
  bc_file = NULL,
  valid_barcode_file = "",
  output_folder = "",
  umi_start = 0,
  umi_length = 0,
  umi_in = "both",
  rmN = FALSE,
  rmlow = FALSE,
  min_qual = 20,
  num_below_min = 2,
  id1_st = -0,
  id1_len = 16,
  id2_st = 0,
  id2_len = 16,
  no_reverse_complement = FALSE
)
```

**Arguments**

r1	read one for pair-end reads.
r2	read two for pair-end reads, NULL if single read.
bc_file	the barcode information, can be either in a fastq format (e.g. from 10x-ATAC) or from a .csv file (here the barcode is expected to be on the second column). Currently, for the fastq approach, this can be a list of barcode files.



<code>valid_barcode_file</code>	optional file path of the valid (expected) barcode sequences to be found in the <code>bc_file</code> (.txt, can be txt.gz). Must contain one barcode per line on the second column separated by a comma (default = ""). If given, each barcode from <code>bc_file</code> is matched against the barcode of best fit (allowing a hamming distance of 1). If a FASTQ <code>bc_file</code> is provided, barcodes with a higher mapping quality, as given by the fastq reads quality score are prioritised.
<code>output_folder</code>	the output dir for the demultiplexed fastq file, which will contain fastq files with reformatted barcode and UMI into the read name. Files ending in .gz will be automatically compressed.
<code>umi_start</code>	if available, the start position of the molecular identifier.
<code>umi_length</code>	if available, the start position of the molecular identifier.
<code>umi_in</code>	<code>umi_in</code>
<code>rmN</code>	logical, whether to remove reads that contains N in UMI or cell barcode.
<code>rmlow</code>	logical, whether to remove reads that have low quality barcode sequences
<code>min_qual</code>	the minimum base pair quality that is allowed (default = 20).
<code>num_below_min</code>	the maximum number of base pairs below the quality threshold.
<code>id1_st</code>	barcode start position (0-indexed) for read 1, which is an extra parameter that is needed if the <code>bc_file</code> is in a .csv format.
<code>id1_len</code>	barcode length for read 1, which is an extra parameter that is needed if the <code>bc_file</code> is in a .csv format.
<code>id2_st</code>	barcode start position (0-indexed) for read 2, which is an extra parameter that is needed if the <code>bc_file</code> is in a .csv format.
<code>id2_len</code>	barcode length for read 2, which is an extra parameter that is needed if the <code>bc_file</code> is in a .csv format.
<code>no_reverse_complement</code>	specifies if the reverse complement of the barcode sequence should be used for barcode error correction (only when barcode sequences are provided as fastq files). FALSE (default) lets the function decide whether to use reverse complement, and TRUE forces the function to use the forward barcode sequences.

**Value**

None (invisible 'NULL')

**Examples**

```
data.folder <- system.file("extdata", package = "scPipe", mustWork = TRUE)
r1      <- file.path(data.folder, "small_chr21_R1.fastq.gz")
r2      <- file.path(data.folder, "small_chr21_R3.fastq.gz")

# Using a barcode fastq file:

# barcodes in fastq format
barcode_fastq <- file.path(data.folder, "small_chr21_R2.fastq.gz")
```

```

sc_atac_trim_barcode (
  r1          = r1,
  r2          = r2,
  bc_file     = barcode_fastq,
  rmN         = TRUE,
  rmlow       = TRUE,
  output_folder = tempdir())

# Using a barcode csv file:

# barcodes in .csv format
barcode_1000 <- file.path(data.folder, "chr21_modified_barcode_1000.csv")

## Not run:
sc_atac_trim_barcode (
  r1          = r1,
  r2          = r2,
  bc_file     = barcode_1000,
  id1_st      = 0,
  rmN         = TRUE,
  rmlow       = TRUE,
  output_folder = tempdir())

## End(Not run)

```

---

```

sc_correct_bam_bc      sc_correct_bam_bc

```

---

## Description

update the cell barcode tag in bam file with corrected barcode output to a new bam file. the function will be useful for methods that use the cell barcode information from bam file, such as ‘Demuxlet’

## Usage

```

sc_correct_bam_bc(
  inbam,
  outbam,
  bc_anno,
  max_mis = 1,
  bam_tags = list(am = "YE", ge = "GE", bc = "BC", mb = "OX"),
  mito = "MT",
  nthreads = 1
)

```

## Arguments

inbam           input bam file. This should be the output of sc\_exon\_mapping  
outbam           output bam file with updated cell barcode

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"> <li>• "am": mapping status tag</li> <li>• "ge": gene id</li> <li>• "bc": cell barcode tag</li> <li>• "mb": molecular barcode tag</li> </ul>
mito	mitochondrial chromosome name. This should be consistent with the chromosome names in the bam file.
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_correct_bam_bc(file.path(data_dir, "out.map.bam"),
  file.path(data_dir, "out.map.clean.bam"),
  barcode_annotation_fn)
...
## End(Not run)

```

---

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,
stnd = 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"> <li>• "am": mapping status tag</li> <li>• "ge": gene id</li> <li>• "bc": cell barcode tag</li> <li>• "mb": molecular barcode tag</li> </ul>
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)
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"> <li>• "am": mapping status tag</li> <li>• "ge": gene id</li> <li>• "bc": cell barcode tag</li> <li>• "mb": molecular barcode tag</li> </ul>
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 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"> <li>• "am": mapping status tag</li> <li>• "ge": gene id</li> <li>• "bc": cell barcode tag</li> <li>• "mb": molecular barcode tag</li> </ul>
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	<i>sc_detect_bc</i>
--------------	---------------------

---

## 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: <a href="https://community.10xgenomics.com/t5/Data-Sharing/List-of-valid-cellular-barcodes/td-p/527">https://community.10xgenomics.com/t5/Data-Sharing/List-of-valid-cellular-barcodes/td-p/527</a> (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

*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>

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"> <li>• "am": mapping status tag</li> <li>• "ge": gene id</li> <li>• "bc": cell barcode tag</li> <li>• "mb": molecular barcode tag</li> </ul>
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\_get\_umap\_data      *Generates UMAP data from sce object*

---

**Description**

Produces a DataFrame containing the UMAP dimensions, as well as all the colData of the sce object for each cell

**Usage**

```
sc_get_umap_data(sce, n_neighbours = 30)
```

**Arguments**

sce	The SingleCellExperiment object
n_neighbours	No. of neighbours for KNN

**Value**

A dataframe containing the UMAP dimensions, as well as all the colData of the sce object for each cell

---

sc_integrate	<i>Integrate multi-omic scRNA-Seq and scATAC-Seq data into a MultiAssayExperiment</i>
--------------	---

---

### Description

Generates an integrated SCE object with scRNA-Seq and scATAC-Seq data produced by the scPipe pipelines

### Usage

```
sc_integrate(
  sce_list,
  barcode_match_file = NULL,
  sce_column_to_barcode_files = NULL,
  rev_comp = NULL,
  cell_line_info = NULL,
  output_folder = NULL
)
```

### Arguments

sce_list	A list of SCE objects, named with the corresponding technologies
barcode_match_file	A .csv file with columns corresponding to the barcodes for each tech
sce_column_to_barcode_files	A list of files containing the barcodes for each tech (if not needed then give a 'NULL' entry)
rev_comp	A named list of technologies and logical flags specifying if reverse complements should be applied for sequences (if not needed then provide a 'NULL' entry)
cell_line_info	A list of files, each of which contains 2 columns corresponding to the barcode and cell line for each tech (if not needed then provide a 'NULL' entry)
output_folder	The path to the output folder

### Value

Returns a MultiAssayExperiment containing the scRNA-Seq and scATAC-Seq data produced by the scPipe pipelines

### Examples

```
## Not run:
sc_integrate(
  sce_list = list("RNA" = sce.rna, "ATAC" = sce.atac),
  barcode_match_file = bc_match_file,
  sce_column_to_barcode_files = list("RNA" = rna_bc_anno, "ATAC" = NULL),
```

```

rev_comp = list("RNA" = FALSE, "ATAC" = TRUE),
cell_line_info = list("RNA" = rna_cell_line_info, "ATAC" = atac_cell_line_info,)
output_folder = output_folder
)

```

```
## End(Not run)
```

---

```
sc_interactive_umap_plot
```

*Produces an interactive UMAP plot via Shiny*

---

### Description

Can colour the UMAP by any of the colData columns in the SCE object

### Usage

```
sc_interactive_umap_plot(sce)
```

### Arguments

sce                    The SingleCellExperiment object

### Value

A shiny object which represents the app. Printing the object or passing it to ‘shiny::runApp(...)’ will run the app.

---

```
sc_mae_plot_umap
```

*Generates UMAP of multiomic data*

---

### Description

Uses feature count data from multiple experiment objects to produce UMAPs for each assay and then overlay them on the same pair of axes

### Usage

```
sc_mae_plot_umap(mae, by = NULL, output_file = NULL)
```

### Arguments

mae                    The MultiAssayExperiment object  
by                      What to colour the points by. Needs to be in colData of all experiments.  
output\_file            The path of the output file

**Value**

A ggplot2 object representing the UMAP plot

---

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.

**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	<i>quality control information for a small sample scRNA-seq dataset to demonstrate capabilities of scPipe.</i>
--------------	--

---

**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.

**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	<i>sc_trim_barcode</i>
-----------------	------------------------

---

**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"> <li>• bs1: starting position of barcode in read one. -1 if no barcode in read one.</li> <li>• 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.</li> <li>• bs2: starting position of barcode in read two</li> <li>• b12: length of barcode in read two</li> <li>• us: starting position of UMI</li> <li>• ul: length of UMI</li> </ul>
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 6bp of the second read followed by a 8bp 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 cell barcode, 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)
```

---

TF.IDF.custom

*Returns the TF-IDF normalised version of a binary matrix*

---

### Description

Returns the TF-IDF normalised version of a binary matrix

### Usage

```
TF.IDF.custom(binary.mat, verbose = TRUE)
```

**Arguments**

binary.mat      The binary matrix  
 verbose        boolean flag to print status messages

**Value**

Returns the TF-IDF normalised version of a binary matrix

---

UMI_duplication	<i>UMI duplication statistics for a small sample scRNA-seq dataset to demonstrate capabilities of scPipe</i>
-----------------	--

---

**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.

**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	<i>Get or set UMI duplication results in a SingleCellExperiment object</i>
--------------	--

---

**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 <a href="#">SingleCellExperiment</a> 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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