

# Package ‘FLAMES’

November 20, 2024

**Title** FLAMES: Full Length Analysis of Mutations and Splicing in long read RNA-seq data

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**Description** Semi-supervised isoform detection and annotation from both bulk and single-cell long read RNA-seq data. Flames provides automated pipelines for analysing isoforms, as well as intermediate functions for manual execution.

**biocViews** RNASeq, SingleCell, Transcriptomics, DataImport, DifferentialSplicing, AlternativeSplicing, GeneExpression, LongRead

**BugReports** <https://github.com/mritchielab/FLAMES/issues>

**License** GPL (>= 3)

**Encoding** UTF-8

**Imports** basilisk, bambu, BiocParallel, Biostrings, BiocGenerics, circlize, ComplexHeatmap, cowplot, dplyr, DropletUtils, GenomicRanges, GenomicFeatures, txdbmaker, GenomicAlignments, GenomeInfoDb, ggplot2, ggbio, grid, gridExtra, igraph, jsonlite, magrittr, Matrix, MatrixGenerics, parallel, readr, reticulate, Rsamtools, rtracklayer, RColorBrewer, SingleCellExperiment, SummarizedExperiment, SpatialExperiment, scater, scatterpie, S4Vectors, scuttle, stats, scan, stringr, tidyr, utils, withr, future, methods, tibble, tidyselect, IRanges

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<i>annotation_to_fasta</i>	<i>GTF/GFF to FASTA conversion</i>
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## Description

convert the transcript annotation to transcriptome assembly as FASTA file. The genome annotation is first imported as TxDb object and then used to extract transcript sequence from the genome assembly.

## Usage

```
annotation_to_fasta(isoform_annotation, genome_fa, outdir, extract_fn)
```

## Arguments

<code>isoform_annotation</code>	Path to the annotation file (GTF/GFF3)
<code>genome_fa</code>	The file path to genome fasta file.
<code>outdir</code>	The path to directory to store the transcriptome as <code>transcript_assembly.fa</code> .
<code>extract_fn</code>	(optional) Function to extract GRangesList from the genome TxDb object. E.g. <code>function(txdb){GenomicFeatures::cdfsBy(txdb, by="tx", use.names=TRUE)}</code>

## Value

Path to the outputted transcriptome assembly

## Examples

```
fasta <- annotation_to_fasta(system.file("extdata", "rps24.gtf.gz", package = "FLAMES"), system.file("extdata", "cat", readChar(fasta, nchars = 1e3))
```

---

blaze	<i>BLAZE Assign reads to cell barcodes.</i>
-------	---

---

### Description

Uses BLAZE to generate barcode list and assign reads to cell barcodes.

### Usage

```
blaze(expect_cells, fq_in, ...)
```

### Arguments

expect_cells	Integer, expected number of cells. Note: this could be just a rough estimate. E.g., the targeted number of cells.
fq_in	File path to the fastq file used as a query sequence file
...	Additional BLAZE configuration parameters. E.g., setting ‘output-prefix’=‘some_prefix’ is equivalent to specifying ‘–output-prefix some_prefix’ in BLAZE; Similarly, ‘overwrite=TRUE’ is equivalent to switch on the ‘–overwrite’ option. Note that the specified parameters will override the parameters specified in the configuration file. All available options can be found at <a href="https://github.com/shimlab/BLAZE">https://github.com/shimlab/BLAZE</a> .

### Value

A data.frame summarising the reads aligned. Other outputs are written to disk. The details of the output files can be found at <https://github.com/shimlab/BLAZE>.

### Examples

```
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
fastq1_url <- 'https://raw.githubusercontent.com/shimlab/BLAZE/main/test/data/FAR20033_pass_51e510db_100.fq'
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, 'Fastq1', fastq1_url))]]
outdir <- tempfile()
dir.create(outdir)
## Not run:
blaze(expect_cells=10, fastq1, overwrite=TRUE)

## End(Not run)
```

---

bulk_long_pipeline	<i>Pipeline for Bulk Data</i>
--------------------	-------------------------------

---

### Description

Semi-supervised isoform detection and annotation for long read data. This variant is meant for bulk samples. Specific parameters relating to analysis can be changed either through function arguments, or through a configuration JSON file.

**Usage**

```
bulk_long_pipeline(
  annotation,
  fastq,
  outdir,
  genome_fa,
  minimap2 = NULL,
  k8 = NULL,
  config_file = NULL
)
```

**Arguments**

annotation	The file path to the annotation file in GFF3 format
fastq	The file path to input fastq file
outdir	The path to directory to store all output files.
genome_fa	The file path to genome fasta file.
minimap2	Path to minimap2, if it is not in PATH. Only required if either or both of do_genome_align and do_read_realign are TRUE.
k8	Path to the k8 Javascript shell binary. Only required if do_genome_align is TRUE.
config_file	File path to the JSON configuration file. If specified, config_file overrides all configuration parameters

**Details**

By default FLAMES use minimap2 for read alignment. After the genome alignment step (do\_genome\_align), FLAMES summarizes the alignment for each read by grouping reads with similar splice junctions to get a raw isoform annotation (do\_isoform\_id). The raw isoform annotation is compared against the reference annotation to correct potential splice site and transcript start/end errors. Transcripts that have similar splice junctions and transcript start/end to the reference transcript are merged with the reference. This process will also collapse isoforms that are likely to be truncated transcripts. If isoform\_id\_bambu is set to TRUE, bambu::bambu will be used to generate the updated annotations. Next is the read realignment step (do\_read\_realign), where the sequence of each transcript from the update annotation is extracted, and the reads are realigned to this updated transcript\_assembly.fa by minimap2. The transcripts with only a few full-length aligned reads are discarded. The reads are assigned to transcripts based on both alignment score, fractions of reads aligned and transcript coverage. Reads that cannot be uniquely assigned to transcripts or have low transcript coverage are discarded. The UMI transcript count matrix is generated by collapsing the reads with the same UMI in a similar way to what is done for short-read scRNA-seq data, but allowing for an edit distance of up to 2 by default. Most of the parameters, such as the minimal distance to splice site and minimal percentage of transcript coverage can be modified by the JSON configuration file (config\_file).

The default parameters can be changed either through the function arguments or through the configuration JSON file config\_file. the pipeline\_parameters section specifies which steps are to be executed in the pipeline - by default, all steps are executed. The isoform\_parameters section affects isoform detection - key parameters include:

Min\_sup\_cnt which causes transcripts with less reads aligned than it's value to be discarded  
 MAX\_TS\_DIST which merges transcripts with the same intron chain and TSS/TES distance less than  
 MAX\_TS\_DIST

`strand_specific` which specifies if reads are in the same strand as the mRNA (1), or the reverse complemented (-1) or not strand specific (0), which results in strand information being based on reference annotation.

### Value

if `do_transcript_quantification` set to true, `bulk_long_pipeline` returns a `SummarizedExperiment` object, containing a count matrix as an assay, gene annotations under metadata, as well as a list of the other output files generated by the pipeline. The pipeline also outputs a number of output files into the given `outdir` directory. These output files generated by the pipeline are:

**transcript\_count.csv.gz** - a transcript count matrix (also contained in the `SummarizedExperiment`)

**isoform\_annotated.filtered.gff3** - isoforms in gff3 format (also contained in the `SummarizedExperiment`)

**transcript\_assembly.fa** - transcript sequence from the isoforms

**align2genome.bam** - sorted BAM file with reads aligned to genome

**realign2transcript.bam** - sorted realigned BAM file using the `transcript_assembly.fa` as reference

**tss\_tes.bedgraph** - TSS TES enrichment for all reads (for QC)

if `do_transcript_quantification` set to false, nothing will be returned

### See Also

[sc\\_long\\_pipeline\(\)](#) for single cell data, [SummarizedExperiment\(\)](#) for how data is outputted

### Examples

```
# download the two fastq files, move them to a folder to be merged together
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <-
  "https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data"
# download the required fastq files, and move them to new folder
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, "Fastq1", paste(file_url, "fastq/sample1.fastq.gz", sep = "/"
fastq2 <- bfc[[names(BiocFileCache::bfcadd(bfc, "Fastq2", paste(file_url, "fastq/sample2.fastq.gz", sep = "/"
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta-
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, "genome.fa", paste(file_url, "SIRV_isoforms_multi-fasta_1
fastq_dir <- paste(temp_path, "fastq_dir", sep = "/") # the downloaded fastq files need to be in a directory to b
dir.create(fastq_dir)
file.copy(c(fastq1, fastq2), fastq_dir)
unlink(c(fastq1, fastq2)) # the original files can be deleted

outdir <- tempfile()
dir.create(outdir)
se <- bulk_long_pipeline(
  annotation = annotation, fastq = fastq_dir, outdir = outdir, genome_fa = genome_fa,
  config_file = create_config(outdir, type = "sc_3end", threads = 1, no_flank = TRUE)
)
```

---

combine_sce	<i>Combine SCE</i>
-------------	--------------------

---

## Description

Combine FLT-seq SingleCellExperiment objects

## Usage

```
combine_sce(sce_with_lr, sce_without_lr)
```

## Arguments

`sce_with_lr` A SingleCellExperiment object with both long and short reads. The long-read transcript counts should be stored in the 'transcript' altExp slot.

`sce_without_lr` A SingleCellExperiment object with only short reads.

## Details

For protocols like FLT-seq that generate two libraries, one with both short and long reads, and one with only short reads, this function combines the two libraries into a single SingleCellExperiment object. For the library with both long and short reads, the long-read transcript counts should be stored in the 'transcript' altExp slot of the SingleCellExperiment object. This function will combine the short-read gene counts of both libraries, and for the transcripts counts, it will leave NA values for the cells from the short-read only library. The `sc_impute_transcript` function can then be used to impute the NA values.

## Value

A SingleCellExperiment object with combined gene counts and a "transcript" altExp slot.

## Examples

```
with_lr <- SingleCellExperiment::SingleCellExperiment(assays = list(counts = matrix(rpois(100, 5), ncol = 10))
without_lr <- SingleCellExperiment::SingleCellExperiment(assays = list(counts = matrix(rpois(200, 5), ncol = 2)
long_read <- SingleCellExperiment::SingleCellExperiment(assays = list(counts = matrix(rpois(50, 5), ncol = 10)
SingleCellExperiment::altExp(with_lr, "transcript") <- long_read
SummarizedExperiment::colData(with_lr)$Barcode <- paste0(1:10, "-1")
SummarizedExperiment::colData(without_lr)$Barcode <- paste0(8:27, "-1")
rownames(with_lr) <- as.character(101:110)
rownames(without_lr) <- as.character(103:112)
rownames(long_read) <- as.character(1001:1005)
combined_sce <- FLAMES::combine_sce(sce_with_lr = with_lr, sce_without_lr = without_lr)
combined_sce
```

---

convolution\_filter      *Convolution filter for smoothing transcript coverages*

---

### Description

Filter out transcripts with sharp drops / rises in coverage, to be used in filter\_coverage to remove transcripts with potential misalignments / internal priming etc. Filtering is done by convolving the coverage with a kernel of 1s and -1s (e.g. c(1, 1, -1, -1), where the width of the 1s and -1s are determined by the width parameter), and check if the maximum absolute value of the convolution is below a threshold. If the convolution is below the threshold, TRUE is returned, otherwise FALSE.

### Usage

```
convolution_filter(x, threshold = 0.15, width = 2, trim = 0.05)
```

### Arguments

x	numeric vector of coverage values
threshold	numeric, the threshold for the maximum absolute value of the convolution
width	numeric, the width of the 1s and -1s in the kernel. E.g. width = 2 will result in a kernel of c(1, 1, -1, -1)
trim	numeric, the proportion of the coverage values to ignore at both ends before convolution.

### Value

logical, TRUE if the transcript passes the filter, FALSE otherwise

### Examples

```
# A >30% drop in coverage will fail the filter with threshold = 0.3
convolution_filter(c(1, 1, 1, 0.69, 0.69, 0.69), threshold = 0.3)
convolution_filter(c(1, 1, 1, 0.71, 0.7, 0.7), threshold = 0.3)
```

---

create\_config      *Create Configuration File From Arguments*

---

### Description

Create Configuration File From Arguments

### Usage

```
create_config(outdir, type = "sc_3end", ...)
```



**Arguments**

outdir	the destination directory for the configuration file
type	use an example config, available values: <b>"sc_3end"</b> - config for 10x 3' end ONT reads <b>"SIRV"</b> - config for the SIRV example reads
...	Configuration parameters. <b>seed</b> - Integer. Seed for minimap2. <b>threads</b> - Number of threads to use. <b>do_barcode_demultiplex</b> - Boolean. Specifies whether to run the barcode demultiplexing step. <b>do_genome_alignment</b> - Boolean. Specifies whether to run the genome alignment step. TRUE is recommended <b>do_gene_quantification</b> - Boolean. Specifies whether to run gene quantification using the genome alignment results. TRUE is recommended <b>do_isoform_identification</b> - Boolean. Specifies whether to run the isoform identification step. TRUE is recommended <b>bambu_isoform_identification</b> - Boolean. Whether to use Bambu for isoform identification. <b>multithread_isoform_identification</b> - Boolean. Whether to use FLAMES' new multithreaded Cpp implementation for isoform identification. <b>do_read_realignment</b> - Boolean. Specifies whether to run the read realignment step. TRUE is recommended <b>do_transcript_quantification</b> - Boolean. Specifies whether to run the transcript quantification step. TRUE is recommended <b>barcode_parameters</b> - List. Parameters for barcode demultiplexing passed to find_barcode (except fastq, barcodes_file, stats_out, reads_out) and threads, which are set by the pipeline, see ?find_barcode for more details. <b>generate_raw_isoform</b> - Boolean. Whether to generate all isoforms for debugging purpose. <b>max_dist</b> - Maximum distance allowed when merging splicing sites in isoform consensus clustering. <b>max_ts_dist</b> - Maximum distance allowed when merging transcript start/end position in isoform consensus clustering. <b>max_splice_match_dist</b> - Maximum distance allowed when merging splice site called from the data and the reference annotation. <b>min_fl_exon_len</b> - Minimum length for the first exon outside the gene body in reference annotation. This is to correct the alignment artifact <b>max_site_per_splice</b> - Maximum transcript start/end site combinations allowed per splice chain <b>min_sup_cnt</b> - Minimum number of read support an isoform decrease this number will significantly increase the number of isoform detected. <b>min_cnt_pct</b> - Minimum percentage of count for an isoform relative to total count for the same gene. <b>min_sup_pct</b> - Minimum percentage of count for an splice chain that support a given transcript start/end site combination. <b>strand_specific</b> - 0, 1 or -1. 1 indicates if reads are in the same strand as mRNA, -1 indicates reads are reverse complemented, 0 indicates reads are not strand specific.

**remove\_incomp\_reads** - The strengde of truncated isoform filtering. larger number means more stringent filtering.

**use\_junctions** - whether to use known splice junctions to help correct the alignment results

**no\_flank** - Boolean. for synthetic spike-in data. refer to Minimapp2 document for detail

**use\_annotation** - Boolean. whether to use reference to help annotate known isoforms

**min\_tr\_coverage** - Minimum percentage of isoform coverage for a read to be aligned to that isoform

**min\_read\_coverage** - Minimum percentage of read coverage for a read to be uniquely aligned to that isoform

### Details

Create a list object containing the arguments supplied in a format usable for the FLAMES pipeline. Also writes the object to a JSON file, which is located with the prefix 'config\_' in the supplied outdir. Default values from extdata/config\_sclr\_nanopore\_3end.json will be used for unprovided parameters.

### Value

file path to the config file created

### Examples

```
# create the default configuration file
outdir <- tempdir()
config <- create_config(outdir)
```

---

create\_sce\_from\_dir    *Create SingleCellExperiment object from FLAMES output folder*

---

### Description

Create SingleCellExperiment object from FLAMES output folder

### Usage

```
create_sce_from_dir(outdir, annotation)
```

### Arguments

outdir            The folder containing FLAMES output files  
 annotation        (Optional) the annotation file that was used to produce the output files

### Value

a list of SingleCellExperiment objects if multiple transcript matrices were found in the output folder, or a SingleCellExperiment object if only one were found

**Examples**

```

outdir <- tempfile()
dir.create(outdir)
bc_allow <- file.path(outdir, "bc_allow.tsv")
genome_fa <- file.path(outdir, "rps24.fa")
R.utils::gunzip(
  filename = system.file("extdata", "bc_allow.tsv.gz", package = "FLAMES"),
  destname = bc_allow, remove = FALSE
)
R.utils::gunzip(
  filename = system.file("extdata", "rps24.fa.gz", package = "FLAMES"),
  destname = genome_fa, remove = FALSE
)
annotation <- system.file("extdata", "rps24.gtf.gz", package = "FLAMES")

sce <- FLAMES::sc_long_pipeline(
  genome_fa = genome_fa,
  fastq = system.file("extdata", "fastq", "muscrps24.fastq.gz", package = "FLAMES"),
  annotation = annotation,
  outdir = outdir,
  barcodes_file = bc_allow,
  config_file = create_config(outdir, oarfish_quantification = FALSE)
)
sce_2 <- create_sce_from_dir(outdir, annotation)

```

---

create\_se\_from\_dir      *Create SummarizedExperiment object from FLAMES output folder*

---

**Description**

Create SummarizedExperiment object from FLAMES output folder

**Usage**

```
create_se_from_dir(outdir, annotation)
```

**Arguments**

outdir                    The folder containing FLAMES output files  
annotation                (Optional) the annotation file that was used to produce the output files

**Value**

a SummarizedExperiment object

**Examples**

```

# download the two fastq files, move them to a folder to be merged together
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <-
  "https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data"
# download the required fastq files, and move them to new folder

```

```

fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, "Fastq1", paste(file_url, "fastq/sample1.fastq.gz", sep = "/"
fastq2 <- bfc[[names(BiocFileCache::bfcadd(bfc, "Fastq2", paste(file_url, "fastq/sample2.fastq.gz", sep = "/"
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta-
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, "genome.fa", paste(file_url, "SIRV_isoforms_multi-fasta_1
fastq_dir <- paste(temp_path, "fastq_dir", sep = "/") # the downloaded fastq files need to be in a directory to b
dir.create(fastq_dir)
file.copy(c(fastq1, fastq2), fastq_dir)
unlink(c(fastq1, fastq2)) # the original files can be deleted

outdir <- tempfile()
dir.create(outdir)
se <- bulk_long_pipeline(
  annotation = annotation, fastq = fastq_dir, outdir = outdir, genome_fa = genome_fa,
  config_file = create_config(outdir, type = "sc_3end", threads = 1, no_flank = TRUE)
)

```

---

create\_spe

*Create a SpatialExperiment object*


---

## Description

This function creates a `SpatialExperiment` object from a `SingleCellExperiment` object and a spatial barcode file.

## Usage

```
create_spe(sce, spatial_barcode_file, manual_align_json, image)
```

## Arguments

sce	The <code>SingleCellExperiment</code> object obtained from running the <a href="#">sc_long_pipeline</a> function.
spatial_barcode_file	The path to the spatial barcode file, e.g. "spaceranger-2.1.1/lib/python/cellranger/barcodes
mannual_align_json	The path to the manual alignment json file.
image	'DataFrame' containing the image data. See <code>?SpatialExperiment::readImgData</code> and <code>?SpatialExperiment::SpatialExperiment</code> .

## Value

A `SpatialExperiment` object.

---

cutadapt	<i>cutadapt wrapper</i>
----------	-------------------------

---

**Description**

trim TSO adaptor with cutadapt

**Usage**

```
cutadapt(args)
```

**Arguments**

args                   arguments to be passed to cutadapt

**Value**

Exit code of cutadapt

**Examples**

```
## Not run:
cutadapt("-h")

## End(Not run)
```

---

demultiplex_sockeye	<i>Demultiplex reads using Sockeye outputs</i>
---------------------	--

---

**Description**

Demultiplex reads using the cell\_umi\_gene.tsv file from Sockeye.

**Usage**

```
demultiplex_sockeye(fastq_dir, sockeye_tsv, out_fq)
```

**Arguments**

fastq_dir	The folder containing FASTQ files from Sockeye's output under ingest/chunked_fastqs.
sockeye_tsv	The cell_umi_gene.tsv file from Sockeye.
out_fq	The output FASTQ file.

**Value**

returns NULL

---

filter_annotation	<i>filter annotation for plotting coverages</i>
-------------------	---

---

### Description

Removes isoform annotations that could produce ambiguous reads, such as isoforms that only differ by the 5' / 3' end. This could be useful for plotting average coverage plots.

### Usage

```
filter_annotation(annotation, keep = "tss_differ")
```

### Arguments

annotation	path to the GTF annotation file, or the parsed GenomicRanges object.
keep	string, one of 'tss_differ' (only keep isoforms that all differ by the transcription start site position), 'tes_differ' (only keep those that differ by the transcription end site position), 'both' (only keep those that differ by both the start and end site), or 'single_transcripts' (only keep genes that contains a single transcript).

### Value

GenomicRanges of the filtered isoforms

### Examples

```
filtered_annotation <- filter_annotation(
  system.file("extdata", "rps24.gtf.gz", package = 'FLAMES'), keep = 'tes_differ')
filtered_annotation
```

---

filter_coverage	<i>Filter transcript coverage</i>
-----------------	-----------------------------------

---

### Description

Filter the transcript coverage by applying a filter function to the coverage values.

### Usage

```
filter_coverage(x, filter_fn = convolution_filter)
```

### Arguments

x	The tibble returned by <a href="#">get_coverage</a> , or a BAM file path, or a GAlignments object.
filter_fn	The filter function to apply to the coverage values. The function should take a numeric vector of coverage values and return a logical value (TRUE if the transcript passes the filter, FALSE otherwise). The default filter function is <a href="#">convolution_filter</a> , which filters out transcripts with sharp drops / rises in coverage.

**Value**

a tibble of the transcript information and coverages, with transcripts that pass the filter

**Examples**

```
# Create a BAM file with minimap2_realign
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <- 'https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data'
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, 'Fastq1', paste(file_url, 'fastq/sample1.fastq.gz', sep = '/')
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, 'genome.fa', paste(file_url, 'SIRV_isoforms_multi-fasta_1
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, 'annot.gtf', paste(file_url, 'SIRV_isoforms_multi-fasta-
outdir <- tempfile()
dir.create(outdir)
fasta <- annotation_to_fasta(annotation, genome_fa, outdir)
minimap2_realign(
  config = jsonlite::fromJSON(
    system.file("extdata", "config_sclr_nanopore_3end.json", package = "FLAMES")),
  fq_in = fastq1,
  outdir = outdir
)
x <- get_coverage(file.path(outdir, 'realign2transcript.bam'))
nrow(x)
filter_coverage(x) |>
  nrow()
```

---

find\_barcode

*Match Cell Barcodes*


---

**Description**

demultiplex reads with flexiplex

**Usage**

```
find_barcode(
  fastq,
  barcodes_file,
  max_bc_editdistance = 2,
  max_flank_editdistance = 8,
  reads_out,
  stats_out,
  threads = 1,
  pattern = c(primer = "CTACACGACGCTCTCCGATCT", BC = paste0(rep("N", 16), collapse =
  "")), UMI = paste0(rep("N", 12), collapse = "")), polyT = paste0(rep("T", 9), collapse
  = "")),
  TSO_seq = "",
  TSO_prime = 3,
  strand = "+",
  full_length_only = FALSE
)
```

**Arguments**

fastq	character vector of paths to FASTQ files or folders, if named, the names will be used as sample names, otherwise the file names will be used
barcodes_file	path to file containing barcode allow-list, with one barcode in each line
max_bc_editdistance	max edit distances for the barcode sequence
max_flank_editdistance	max edit distances for the flanking sequences (primer and polyT)
reads_out	path to output FASTQ file; if multiple samples are processed, the sample name will be appended to this argument, e.g. provide path/out.fq for single sample, and path/prefix for multiple samples.
stats_out	path of output stats file; similar to reads_out, e.g. provide path/stats.tsv for single sample, and path/prefix for multiple samples.
threads	number of threads to be used
pattern	named character vector defining the barcode pattern
TSO_seq	TSO sequence to be trimmed
TSO_prime	either 3 (when TSO_seq is on 3' the end) or 5 (on 5' end)
strand	strand of the barcode pattern, either '+' or '-' (read will be reverse complemented after barcode matching if '-')
full_length_only	boolean, when TSO sequence is provided, whether reads without TSO are to be discarded

**Details**

This function demultiplexes reads by searching for flanking sequences (adaptors) around the barcode sequence, and then matching against allowed barcodes. For single sample, either provide a single FASTQ file or a folder containing FASTQ files. For multiple samples, provide a vector of paths (either to FASTQ files or folders containing FASTQ files). Gzipped file input are supported but the output will be uncompressed.

**Value**

a list containing: reads\_tb (tibble of read demultiplexed information) and input, output, read1\_with\_adapter from cutadapt report (if TSO trimming is performed)

**Examples**

```
outdir <- tempfile()
dir.create(outdir)
bc_allow <- file.path(outdir, "bc_allow.tsv")
R.utils::gunzip(
  filename = system.file("extdata", "bc_allow.tsv.gz", package = "FLAMES"),
  destname = bc_allow, remove = FALSE
)
# single sample
find_barcode(
  fastq = system.file("extdata", "fastq", "muscrps24.fastq.gz", package = "FLAMES"),
  stats_out = file.path(outdir, "bc_stat"),
  reads_out = file.path(outdir, "demultiplexed.fq"),
```



```
    barcodes_file = bc_allow
  )
  # multi-sample
  fastq_dir <- tempfile()
  dir.create(fastq_dir)
  file.copy(system.file("extdata", "fastq", "muscrps24.fastq.gz", package = "FLAMES"),
    file.path(fastq_dir, "muscrps24.fastq.gz"))
  sampled_lines <- readLines(file.path(fastq_dir, "muscrps24.fastq.gz"), n = 400)
  writelines(sampled_lines, file.path(fastq_dir, "copy.fastq"))
  result <- find_barcode(
    # you can mix folders and files. each path will be considered as a sample
    fastq = c(fastq_dir, system.file("extdata", "fastq", "muscrps24.fastq.gz", package = "FLAMES")),
    stats_out = file.path(outdir, "bc_stat"),
    reads_out = file.path(outdir, "demultiplexed.fq"),
    barcodes_file = bc_allow, TSO_seq = "CCCATGACTCTGCGTTGATACCACTGCTT"
  )
```

---

find\_bin

*Find path to a binary Wrapper for Sys.which to find path to a binary*

---

## Description

This function is a wrapper for `base::Sys.which` to find the path to a command. It also searches within the FLAMES basilisk conda environment. This function also replaces "" with NA in the output of `base::Sys.which` to make it easier to check if the binary is found.

## Usage

```
find_bin(command)
```

## Arguments

command            character, the command to search for

## Value

character, the path to the command or NA

## Examples

```
find_bin("minimap2")
```

---

find_isoform	<i>Isoform identification</i>
--------------	-------------------------------

---

## Description

Long-read isoform identification with FLAMES or bambu.

## Usage

```
find_isoform(annotation, genome_fa, genome_bam, outdir, config)
```

## Arguments

annotation	Path to annotation file. If configured to use bambu, the annotation must be provided as GTF file.
genome_fa	The file path to genome fasta file.
genome_bam	File path to BAM alignment file. Multiple files could be provided.
outdir	The path to directory to store all output files.
config	Parsed FLAMES configurations.

## Value

The updated annotation and the transcriptome assembly will be saved in the output folder as isoform\_annotated.gff3 (GTF if bambu is selected) and transcript\_assembly.fa respectively.

## Examples

```
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <- "https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data"
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, "Fastq1", paste(file_url, "fastq/sample1.fastq.gz", sep = "/"
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, "genome.fa", paste(file_url, "SIRV_isoforms_multi-fasta_1
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta-
outdir <- tempfile()
dir.create(outdir)
config <- jsonlite::fromJSON(
  system.file("extdata", "config_sclr_nanopore_3end.json", package = "FLAMES")
)
minimap2_align(
  config = config,
  fa_file = genome_fa,
  fq_in = fastq1,
  annot = annotation,
  outdir = outdir
)
## Not run:
find_isoform(
  annotation = annotation, genome_fa = genome_fa,
  genome_bam = file.path(outdir, "align2genome.bam"),
  outdir = outdir, config = config
)

## End(Not run)
```

---

find_variants	<i>bulk variant identification</i>
---------------	------------------------------------

---

## Description

Treat each bam file as a bulk sample and identify variants against the reference

## Usage

```
find_variants(
  bam_path,
  reference,
  annotation,
  min_nucleotide_depth = 100,
  homopolymer_window = 3,
  annotated_region_only = FALSE,
  names_from = "gene_name",
  threads = 1
)
```

## Arguments

bam_path	character(1) or character(n): path to the bam file(s) aligned to the reference genome (NOT the transcriptome!).
reference	DNAStrngSet: the reference genome
annotation	GRanges: the annotation of the reference genome. You can load a GTF/GFF annotation file with <code>anno &lt;- rtracklayer::import(file)</code> .
min_nucleotide_depth	integer(1): minimum read depth for a position to be considered a variant.
homopolymer_window	integer(1): the window size to calculate the homopolymer percentage. The homopolymer percentage is calculated as the percentage of the most frequent nucleotide in a window of <code>-homopolymer_window</code> to <code>homopolymer_window</code> nucleotides around the variant position, excluding the variant position itself. Calculation of the homopolymer percentage is skipped when <code>homopolymer_window = 0</code> . This is useful for filtering out Nanopore sequencing errors in homopolymer regions.
annotated_region_only	logical(1): whether to only consider variants outside annotated regions. If TRUE, only variants outside annotated regions will be returned. If FALSE, all variants will be returned, which could take significantly longer time.
names_from	character(1): the column name in the metadata column of the annotation ( <code>mcols(annotation)[, names_from]</code> ) to use for the region column in the output.
threads	integer(1): number of threads to use. Threading is done over each annotated region and (if <code>annotated_region_only = FALSE</code> ) unannotated gaps for each bam file.

**Details**

Each bam file is treated as a bulk sample to perform pileup and identify variants. You can run `sc_mutations` with the variants identified with this function to get single-cell allele counts. Note that reference genome FASTA files may have the chromosome names field as '>chr1 1' instead of '>chr1'. You may need to remove the trailing number to match the chromosome names in the bam file, for example with `names(ref) <- sapply(names(ref), function(x) strsplit(x, " ")[[1]][1])`.

**Value**

A tibble with columns: `seqnames`, `pos`, `nucleotide`, `count`, `sum`, `freq`, `ref`, `region`, `homopolymer_pct`, `bam_path` The homopolymer percentage is calculated as the percentage of the most frequent nucleotide in a window of `homopolymer_window` nucleotides around the variant position, excluding the variant position itself.

**Examples**

```
outdir <- tempfile()
dir.create(outdir)
genome_fa <- system.file("extdata", "rps24.fa.gz", package = "FLAMES")
minimap2_align( # align to genome
  config = jsonlite::fromJSON(
    system.file("extdata", "config_sclr_nanopore_3end.json", package = "FLAMES")),
  fa_file = genome_fa,
  fq_in = system.file("extdata", "fastq", "demultiplexed.fq.gz", package = "FLAMES"),
  annot = system.file("extdata", "rps24.gtf.gz", package = "FLAMES"),
  outdir = outdir
)
variants <- find_variants(
  bam_path = file.path(outdir, "align2genome.bam"),
  reference = genome_fa,
  annotation = system.file("extdata", "rps24.gtf.gz", package = "FLAMES"),
  min_nucleotide_depth = 4
)
head(variants)
```

---

 FLAMES

---

*FLAMES: full-length analysis of mutations and splicing*


---

**Description**

FLAMES: full-length analysis of mutations and splicing

---

`flexiplex`*Rcpp port of flexiplex*

---

### Description

demultiplex reads with flexiplex, for detailed description, see documentation for the original flexiplex: <https://davidsongroup.github.io/flexiplex>

### Usage

```
flexiplex(  
  reads_in,  
  barcodes_file,  
  bc_as_readid,  
  max_bc_editdistance,  
  max_flank_editdistance,  
  pattern,  
  reads_out,  
  stats_out,  
  bc_out,  
  reverseCompliment,  
  n_threads  
)
```

### Arguments

<code>reads_in</code>	Input FASTQ or FASTA file
<code>barcodes_file</code>	barcode allow-list file
<code>bc_as_readid</code>	bool, whether to add the demultiplexed barcode to the read ID field
<code>max_bc_editdistance</code>	max edit distance for barcode '
<code>max_flank_editdistance</code>	max edit distance for the flanking sequences '
<code>pattern</code>	StringVector defining the barcode structure, see [find_barcode]
<code>reads_out</code>	output file for demultiplexed reads
<code>stats_out</code>	output file for demultiplexed stats
<code>bc_out</code>	WIP
<code>reverseCompliment</code>	bool, whether to reverse complement the reads after demultiplexing
<code>n_threads</code>	number of threads to be used during demultiplexing

### Value

integer return value. 0 represents normal return.

---

get\_coverage *Get read coverages from BAM file*

---

### Description

Get the read coverages for each transcript in the BAM file (or a GAlignments object). The read coverages are sampled at 100 positions along the transcript, and the coverage is scaled by dividing the coverage at each position by the total read counts for the transcript. If a BAM file is provided, alignment with MAPQ < 5, secondary alignments and supplementary alignments are filtered out. A GAlignments object can also be provided in case alternative filtering is desired.

### Usage

```
get_coverage(bam, min_counts = 10, remove_UTR = FALSE, annotation)
```

### Arguments

bam	path to the BAM file, or a parsed GAlignments object
min_counts	numeric, the minimum number of alignments required for a transcript to be included
remove_UTR	logical, if TRUE, remove the UTRs from the coverage
annotation	(Required if remove_UTR = TRUE) path to the GTF annotation file

### Value

a tibble of the transcript information and coverages, with the following columns:

- transcript: the transcript name / ID
- read\_counts: the total number of alignments for the transcript
- coverage\_1-100: the coverage at each of the 100 positions along the transcript
- tr\_length: the length of the transcript

### Examples

```
# Create a BAM file with minimap2_realign
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <- 'https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data'
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, 'Fastq1', paste(file_url, 'fastq/sample1.fastq.gz', sep = '/')
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, 'genome.fa', paste(file_url, 'SIRV_isoforms_multi-fasta_1
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, 'annot.gtf', paste(file_url, 'SIRV_isoforms_multi-fasta-
outdir <- tempfile()
dir.create(outdir)
fasta <- annotation_to_fasta(annotation, genome_fa, outdir)
minimap2_realign(
  config = jsonlite::fromJSON(
    system.file("extdata", "config_sclr_nanopore_3end.json", package = "FLAMES")),
  fq_in = fastq1,
  outdir = outdir
)
x <- get_coverage(file.path(outdir, 'realign2transcript.bam'))
head(x)
```

---

get_GRangesList	<i>Parse FLAMES' GFF output</i>
-----------------	---------------------------------

---

## Description

Parse FLAMES' GFF outputs into a Genomic Ranges List

## Usage

```
get_GRangesList(file)
```

## Arguments

file                    the GFF file to parse

## Value

A Genomic Ranges List

## Examples

```
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <- "https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data"
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, "Fastq1", paste(file_url, "fastq/sample1.fastq.gz", sep = "/"))
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, "genome.fa", paste(file_url, "SIRV_isoforms_multi-fasta_1
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta-
outdir <- tempfile()
dir.create(outdir)
config <- jsonlite::fromJSON(
  system.file("extdata", "config_sclr_nanopore_3end.json", package = "FLAMES"))
minimap2_align(
  config = config,
  fa_file = genome_fa,
  fq_in = fastq1,
  annot = annotation,
  outdir = outdir
)
find_isoform(
  annotation = annotation, genome_fa = genome_fa,
  genome_bam = file.path(outdir, "align2genome.bam"),
  outdir = outdir, config = config
)
grlist <- get_GRangesList(file = file.path(outdir, "isoform_annotated.gff3"))
```

---

 minimap2\_align

*Minimap2 Align to Genome*


---

### Description

Uses minimap2 to align sequences against a reference database. Uses options '-ax splice -t 12 -k14 --secondary=no fa\_file fq\_in'

### Usage

```
minimap2_align(
  config,
  fa_file,
  fq_in,
  annot,
  outdir,
  minimap2 = NA,
  k8 = NA,
  samtools = NA,
  prefix = NULL,
  threads = 1
)
```

### Arguments

config	Parsed list of FLAMES config file
fa_file	Path to the fasta file used as a reference database for alignment
fq_in	File path to the fastq file used as a query sequence file
annot	Genome annotation file used to create junction bed files
outdir	Output folder
minimap2	Path to minimap2 binary
k8	Path to the k8 Javascript shell binary
samtools	path to the samtools binary, required for large datasets since Rsamtools does not support CSI indexing
prefix	String, the prefix (e.g. sample name) for the outputted BAM file
threads	Integer, threads for minimap2 to use, see minimap2 documentation for details, FLAMES will try to detect cores if this parameter is not provided.

### Value

a data.frame summarising the reads aligned

### See Also

[minimap2\_realign()]



**Examples**

```

temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <- 'https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data'
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, 'Fastq1', paste(file_url, 'fastq/sample1.fastq.gz', sep = '/')
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, 'genome.fa', paste(file_url, 'SIRV_isoforms_multi-fasta_1
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, 'annot.gtf', paste(file_url, 'SIRV_isoforms_multi-fasta-
outdir <- tempfile()
dir.create(outdir)
minimap2_align(
  config = jsonlite::fromJSON(
    system.file("extdata", "config_sclr_nanopore_3end.json", package = 'FLAMES')
  ),
  fa_file = genome_fa,
  fq_in = fastq1,
  annot = annotation,
  outdir = outdir
)

```

minimap2\_realign

*Minimap2 re-align reads to transcriptome***Description**

Uses minimap2 to re-align reads to transcriptome

**Usage**

```

minimap2_realign(
  config,
  fq_in,
  outdir,
  minimap2,
  samtools = NULL,
  prefix = NULL,
  minimap2_args,
  sort_by,
  threads = 1
)

```

**Arguments**

config	Parsed list of FLAMES config file
fq_in	File path to the fastq file used as a query sequence file
outdir	Output folder
minimap2	Path to minimap2 binary
samtools	path to the samtools binary, required for large datasets since Rsamtools does not support CSI indexing
prefix	String, the prefix (e.g. sample name) for the outputted BAM file
minimap2_args	vector of command line arguments to pass to minimap2

sort_by	String, If provided, sort the BAM file by this tag instead of by position.
threads	Integer, threads for minimap2 to use, see minimap2 documentation for details, FLAMES will try to detect cores if this parameter is not provided.

**Value**

a data.frame summarising the reads aligned

**See Also**

[minimap2\_align()]

**Examples**

```
outdir <- tempfile()
dir.create(outdir)
annotation <- system.file('extdata', 'rps24.gtf.gz', package = 'FLAMES')
genome_fa <- system.file('extdata', 'rps24.fa.gz', package = 'FLAMES')
fasta <- annotation_to_fasta(annotation, genome_fa, outdir)
fastq <- system.file('extdata', 'fastq', 'demultiplexed.fq.gz', package = 'FLAMES')
minimap2_realign(
  config = jsonlite::fromJSON(
    system.file("extdata", "config_sclr_nanopore_3end.json", package = 'FLAMES')
  ),
  fq_in = fastq,
  outdir = outdir
)
```

---

parse\_gff\_tree

*Parse Gff3 file*

---

**Description**

Parse a Gff3 file into 3 components: chromosome to gene name, a transcript dictionary, a gene to transcript dictionary and a transcript to exon dictionary. These components are returned in a named list.

**Usage**

```
parse_gff_tree(gff_file)
```

**Arguments**

gff\_file            the file path to the gff3 file to parse

**Value**

a named list with the elements "chr\_to\_gene", "transcript\_dict", "gene\_to\_transcript", "transcript\_to\_exon", containing the data parsed from the gff3 file.

**Examples**

```
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <-
  "https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data"
gff <- bfc[[names(BiocFileCache::bfcadd(bfc, "GFF", paste(file_url, "SIRV_isoforms_multi-fasta-annotation_C_
## Not run: parsed_gff <- parse_gff_tree(gff)
```

---

plot_coverage	<i>plot read coverages</i>
---------------	----------------------------

---

**Description**

Plot the average read coverages for each length bin or a particular isoform

**Usage**

```
plot_coverage(
  x,
  quantiles = c(0, 0.2375, 0.475, 0.7125, 0.95, 1),
  length_bins = c(0, 1, 2, 5, 10, Inf),
  weight_fn = weight_transcripts,
  filter_fn,
  detailed = FALSE
)
```

**Arguments**

x	path to the BAM file (aligning reads to the transcriptome), or the (GenomicAlignments::readGAlignments) parsed GAlignments object, or the tibble returned by <a href="#">get_coverage</a> , or the filtered tibble returned by <a href="#">filter_coverage</a> .
quantiles	numeric vector to specify the quantiles to bin the transcripts lengths by if length_bins is missing. The length bins will be determined such that the read counts are distributed according to the quantiles.
length_bins	numeric vector to specify the sizes to bin the transcripts by
weight_fn	function to calculate the weights for the transcripts. The function should take a numeric vector of read counts and return a numeric vector of weights. The default function is <a href="#">weight_transcripts</a> , you can change its default parameters by passing an anonymous function like function(x) weight_transcripts(x, type = 'equal').
filter_fn	Optional filter function to filter the transcripts before plotting. See the filter_fn parameter in <a href="#">filter_coverage</a> for more details. Providing a filter function here is the same as providing it in <a href="#">filter_coverage</a> and then passing the result to this function.
detailed	logical, if TRUE, also plot the top 10 transcripts with the highest read counts for each length bin.

**Value**

a ggplot2 object of the coverage plot(s)

## Examples

```
# Create a BAM file with minimap2_realign
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <- 'https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data'
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, 'Fastq1', paste(file_url, 'fastq/sample1.fastq.gz', sep = '/')
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, 'genome.fa', paste(file_url, 'SIRV_isoforms_multi-fasta_1
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, 'annot.gtf', paste(file_url, 'SIRV_isoforms_multi-fasta-
outdir <- tempfile()
dir.create(outdir)
fasta <- annotation_to_fasta(annotation, genome_fa, outdir)
minimap2_realign(
  config = jsonlite::fromJSON(
    system.file("extdata", "config_sclr_nanopore_3end.json", package = "FLAMES")),
  fq_in = fastq1,
  outdir = outdir
)
# Plot the coverages directly from the BAM file
plot_coverage(file.path(outdir, 'realign2transcript.bam'))

# Get the coverage information first
coverage <- get_coverage(file.path(outdir, 'realign2transcript.bam')) |>
  dplyr::filter(read_counts > 2) |> # Filter out transcripts with read counts < 3
  filter_coverage(filter_fn = convolution_filter) # Filter out transcripts with sharp drops / rises
# Plot the filtered coverages
plot_coverage(coverage, detailed = TRUE)
# filtering function can also be passed directly to plot_coverage
plot_coverage(file.path(outdir, 'realign2transcript.bam'), filter_fn = convolution_filter)
```

---

plot\_demultiplex

*Plot Cell Barcode demultiplex statistics*

---

## Description

produce a barplot of cell barcode demultiplex statistics

## Usage

```
plot_demultiplex(find_barcode_result)
```

## Arguments

find\_barcode\_result  
output from [find\\_barcode](#)

## Value

a list of ggplot objects:

- reads\_count\_plot: stacked barplot of: demultiplexed reads
- knee\_plot: knee plot of UMI counts before TSO trimming
- flank\_editdistance\_plot: flanking sequence (adaptor) edit-distance plot
- barcode\_editdistance\_plot: barcode edit-distance plot
- cutadapt\_plot: if TSO trimming is performed, number of reads kept by cutadapt

**Examples**

```

outdir <- tempfile()
dir.create(outdir)
fastq_dir <- tempfile()
dir.create(fastq_dir)
file.copy(system.file("extdata", "fastq", "muscrps24.fastq.gz", package = "FLAMES"),
  file.path(fastq_dir, "muscrps24.fastq.gz"))
sampled_lines <- readLines(file.path(fastq_dir, "muscrps24.fastq.gz"), n = 400)
writelines(sampled_lines, file.path(fastq_dir, "copy.fastq"))
bc_allow <- file.path(outdir, "bc_allow.tsv")
R.utils::gunzip(
  filename = system.file("extdata", "bc_allow.tsv.gz", package = "FLAMES"),
  destname = bc_allow, remove = FALSE
)
find_barcode(
  fastq = fastq_dir,
  stats_out = file.path(outdir, "bc_stat"),
  reads_out = file.path(outdir, "demultiplexed.fq"),
  barcodes_file = bc_allow, TSO_seq = "CCCATGTA CTGCGTTGATACCACTGCTT"
) |>
plot_demultiplex()

```

plot\_isoforms

*Plot isoforms***Description**

Plot isoforms, either from a gene or a list of transcript ids.

**Usage**

```

plot_isoforms(
  sce,
  gene_id,
  transcript_ids,
  n = 4,
  format = "plot_grid",
  colors
)

```

**Arguments**

sce	The SingleCellExperiment object containing transcript counts, rowRanges and rowData with gene_id and transcript_id columns.
gene_id	The gene symbol of interest, ignored if transcript_ids is provided.
transcript_ids	The transcript ids to plot.
n	The number of top isoforms to plot from the gene. Ignored if transcript_ids is provided.
format	The format of the output, either "plot_grid" or "list".
colors	A character vector of colors to use for the isoforms. If not provided, gray will be used. for all isoforms.

**Details**

This function takes a `SingleCellExperiment` object and plots the top isoforms of a gene, or a list of specified transcript ids. Either as a list of plots or together in a grid. This function wraps the `ggbio::geom_alignment` function to plot the isoforms, and orders the isoforms by expression levels (when specifying a gene) or by the order of the `transcript_ids`.

**Value**

When `format = "list"`, a list of `ggplot` objects is returned. Otherwise, a grid of the plots is returned.

**Examples**

```
plot_isoforms(scmixology_lib10_transcripts, gene_id = "ENSG00000108107")
```

---

plot\_isoform\_heatmap *FLAMES heatmap plots*

---

**Description**

Plot expression heatmap of top n isoforms of a gene

**Usage**

```
plot_isoform_heatmap(
  sce,
  gene_id,
  transcript_ids,
  n = 4,
  isoform_legend_width = 7,
  col_low = "#313695",
  col_mid = "#FFFFBF",
  col_high = "#A50026",
  color_quantile = 1
)
```

**Arguments**

<code>sce</code>	The <code>SingleCellExperiment</code> object containing transcript counts, <code>rowRanges</code> and <code>rowData</code> with <code>gene_id</code> and <code>transcript_id</code> columns.
<code>gene_id</code>	The gene symbol of interest, ignored if <code>transcript_ids</code> is provided.
<code>transcript_ids</code>	The transcript ids to plot.
<code>n</code>	The number of top isoforms to plot from the gene. Ignored if <code>transcript_ids</code> is provided.
<code>isoform_legend_width</code>	The width of isoform legends in heatmaps, in cm.
<code>col_low</code>	Color for cells with low expression levels in UMAPs.
<code>col_mid</code>	Color for cells with intermediate expression levels in UMAPs.

`col_high` Color for cells with high expression levels in UMAPs.

`color_quantile` The lower and upper expression quantile to be displayed between `col_low` and `col_high`, e.g. with `color_quantile = 0.95`, cells with expressions higher than 95% of other cells will all be shown in `col_high`, and cells with expression lower than 95% of other cells will all be shown in `col_low`.

### Details

Takes `SingleCellExperiment` object and plots an expression heatmap with the isoform visualizations along genomic coordinates.

### Value

a `ComplexHeatmap`

### Examples

```
scmixology_lib10_transcripts |>
  scuttle::logNormCounts() |>
  plot_isoform_heatmap(gene = "ENSG00000108107")
```

---

`plot_isoform_reduced_dim`

*FLAMES isoform reduced dimensions plots*

---

### Description

Plot expression of top n isoforms of a gene in reduced dimensions

### Usage

```
plot_isoform_reduced_dim(
  sce,
  gene_id,
  transcript_ids,
  n = 4,
  reduced_dim_name = "UMAP",
  use_gene_dimred = FALSE,
  expr_func = function(x) {
    SingleCellExperiment::logcounts(x)
  },
  col_low = "#313695",
  col_mid = "#FFFFBF",
  col_high = "#A50026",
  color_quantile = 1,
  format = "plot_grid",
  ...
)
```

**Arguments**

sce	The SingleCellExperiment object containing transcript counts, rowRanges and rowData with gene_id and transcript_id columns.
gene_id	The gene symbol of interest, ignored if transcript_ids is provided.
transcript_ids	The transcript ids to plot.
n	The number of top isoforms to plot from the gene. Ignored if transcript_ids is provided.
reduced_dim_name	The name of the reduced dimension to use for plotting cells.
use_gene_dimred	Whether to use gene-level reduced dimensions for plotting. Set to TRUE if the SingleCellExperiment has gene counts in main assay and transcript counts in altExp.
expr_func	The function to extract expression values from the SingleCellExperiment object. Default is logcounts. Alternatively, counts can be used for raw counts.
col_low	Color for cells with low expression levels in UMAPs.
col_mid	Color for cells with intermediate expression levels in UMAPs.
col_high	Color for cells with high expression levels in UMAPs.
color_quantile	The lower and upper expression quantile to be displayed between col_low and col_high, e.g. with color_quantile = 0.95, cells with expressions higher than 95% of other cells will all be shown in col_high, and cells with expression lower than 95% of other cells will all be shown in col_low.
format	The format of the output, either "plot_grid" or "list".
...	Additional arguments to pass to plot_grid.

**Details**

Takes SingleCellExperiment object and plots an expression on reduced dimensions with the isoform visualizations along genomic coordinates.

**Value**

a ggplot object of the UMAP(s)

**Examples**

```
scmixology_lib10 <-
  scmixology_lib10[, colSums(SingleCellExperiment::counts(scmixology_lib10)) > 0]
sce_lr <- scmixology_lib10[, colnames(scmixology_lib10) %in% colnames(scmixology_lib10_transcripts)]
SingleCellExperiment::altExp(sce_lr, "transcript") <-
  scmixology_lib10_transcripts[, colnames(sce_lr)]
combined_sce <- combine_sce(sce_lr, scmixology_lib90)
combined_sce <- combined_sce |>
  scuttle::logNormCounts() |>
  scater::runPCA() |>
  scater::runUMAP()
combined_imputed_sce <- sc_impute_transcript(combined_sce)
plot_isoform_reduced_dim(combined_sce, 'ENSG00000108107')
plot_isoform_reduced_dim(combined_imputed_sce, 'ENSG00000108107')
```



---

plot\_spatial\_isoform *Plot spatial pie chart of isoforms*

---

**Description**

This function plots a spatial pie chart for given features.

**Usage**

```
plot_spatial_isoform(spe, isoforms, assay_type = "counts")
```

**Arguments**

spe                    The SpatialExperiment object.  
isoforms              The isoforms to plot.  
assay\_type            The assay that contains the given features. E.g. 'counts', 'logcounts'.

**Value**

A ggplot object.

---

plot\_spatial\_pie *Plot spatial pie chart*

---

**Description**

This function plots a spatial pie chart for given features.

**Usage**

```
plot_spatial_pie(spe, features, assay_type = "counts")
```

**Arguments**

spe                    The SpatialExperiment object.  
features              The features to plot.  
assay\_type            The assay that contains the given features.

**Value**

A ggplot object.

---

quantify_gene	<i>Gene quantification</i>
---------------	----------------------------

---

### Description

Calculate the per gene UMI count matrix by parsing the genome alignment file.

### Usage

```
quantify_gene(
  annotation,
  outdir,
  infq,
  n_process,
  pipeline = "sc_single_sample",
  samples = NULL,
  random_seed = 2024
)
```

### Arguments

annotation	The file path to the annotation file in GFF3 format
outdir	The path to directory to store all output files.
infq	The input FASTQ file.
n_process	The number of processes to use for parallelization.
pipeline	The pipeline type as a character string, either <code>sc_single_sample</code> (single-cell, single-sample),
samples	A vector of sample names, default to the file names of input fastq files, or folder names if <code>fastqs</code> is a vector of folders. <code>bulk</code> (bulk, single or multi-sample), or <code>sc_multi_sample</code> (single-cell, multiple samples)
random_seed	The random seed for reproducibility.

### Details

After the genome alignment step (`do_genome_align`), the alignment file will be parsed to generate the per gene UMI count matrix. For each gene in the annotation file, the number of reads overlapping with the gene's genomic coordinates will be assigned to that gene. If a read overlaps multiple genes, it will be assigned to the gene with the highest number of overlapping nucleotides. If exon coordinates are included in the provided annotation, the decision will first consider the number of nucleotides aligned to the exons of each gene. In cases of a tie, the overlap with introns will be used as a tiebreaker. If there is still a tie after considering both exons and introns, a random gene will be selected from the tied candidates.

After the read-to-gene assignment, the per gene UMI count matrix will be generated. Specifically, for each gene, the reads with similar mapping coordinates of transcript termination sites (TTS, i.e. the end of the the read with a polyT or polyA) will be grouped together. UMIs of reads in the same group will be collapsed to generate the UMI counts for each gene.

Finally, a new fastq file with deduplicated reads by keeping the longest read in each UMI.

**Value**

The count matrix will be saved in the output folder as `transcript_count.csv.gz`.

---

`quantify_transcript`     *Transcript quantification*

---

**Description**

Calculate the transcript count matrix by parsing the re-alignment file.

**Usage**

```
quantify_transcript(
  annotation,
  outdir,
  config,
  pipeline = "sc_single_sample",
  ...
)
```

**Arguments**

<code>annotation</code>	The file path to the annotation file in GFF3 format
<code>outdir</code>	The path to directory to store all output files.
<code>config</code>	Parsed FLAMES configurations.
<code>pipeline</code>	The pipeline type as a character string, either <code>sc_single_sample</code> (single-cell, single-sample),
<code>...</code>	Supply sample names as character vector (e.g. <code>samples = c("name1", "name2", ...)</code> ) for multi-sample or bulk pipeline. <code>bulk</code> (bulk, single or multi-sample), or <code>sc_multi_sample</code> (single-cell, multiple samples)

**Value**

A `SingleCellExperiment` object for single-cell pipeline, a list of `SingleCellExperiment` objects for multi-sample pipeline, or a `SummarizedExperiment` object for bulk pipeline.

**Examples**

```
temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <- "https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data"
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, "Fastq1", paste(file_url, "fastq/sample1.fastq.gz", sep = "/"
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, "genome.fa", paste(file_url, "SIRV_isoforms_multi-fasta_1
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta_
outdir <- tempfile()
dir.create(outdir)
fasta <- annotation_to_fasta(annotation, genome_fa, outdir)
config <- jsonlite::fromJSON(create_config(outdir, bambu_isoform_identification = TRUE, min_tr_coverage = 0.1
file.copy(annotation, file.path(outdir, "isoform_annotated.gtf"))
## Not run:
if (!any(is.na(find_bin(c("minimap2", "k8"))))) {
```

```

minimap2_realign(
  config = config, outdir = outdir,
  fq_in = fastq1
)
quantify_transcript_flames(annotation, outdir, config, pipeline = "bulk")
}

## End(Not run)

```

---

quantify\_transcript\_flames

*FLAMES Transcript quantification*

---

## Description

Calculate the transcript count matrix by parsing the re-alignment file.

## Usage

```

quantify_transcript_flames(
  annotation,
  outdir,
  config,
  pipeline = "sc_single_sample",
  samples
)

```

## Arguments

annotation	The file path to the annotation file in GFF3 format
outdir	The path to directory to store all output files.
config	Parsed FLAMES configurations.
pipeline	The pipeline type as a character string, either <code>sc_single_sample</code> (single-cell, single-sample),
samples	A vector of sample names, required for <code>sc_multi_sample</code> pipeline. <code>bulk</code> (bulk, single or multi-sample), or <code>sc_multi_sample</code> (single-cell, multiple samples)

## Value

A `SingleCellExperiment` object for single-cell pipeline, a list of `SingleCellExperiment` objects for multi-sample pipeline, or a `SummarizedExperiment` object for bulk pipeline.

## Examples

```

temp_path <- tempfile()
bfc <- BiocFileCache::BiocFileCache(temp_path, ask = FALSE)
file_url <- "https://raw.githubusercontent.com/OliverVoogd/FLAMESData/master/data"
fastq1 <- bfc[[names(BiocFileCache::bfcadd(bfc, "Fastq1", paste(file_url, "fastq/sample1.fastq.gz", sep = "/"))
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, "genome.fa", paste(file_url, "SIRV_isoforms_multi-fasta_1
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta_
outdir <- tempfile()

```

```

dir.create(outdir)
fasta <- annotation_to_fasta(annotation, genome_fa, outdir)
config <- jsonlite::fromJSON(create_config(outdir, bambu_isoform_identification = TRUE, min_tr_coverage = 0.1))
file.copy(annotation, file.path(outdir, "isoform_annotated.gtf"))
## Not run:
if (!any(is.na(find_bin(c("minimap2", "k8"))))) {
  minimap2_realign(
    config = config, outdir = outdir,
    fq_in = fastq1
  )
  quantify_transcript_flames(annotation, outdir, config, pipeline = "bulk")
}

## End(Not run)

```

---

```
relative_mutation_positions
```

*Relative mutation positions within the gene body*

---

## Description

Given a set of mutations and gene annotation, calculate the relative position of each mutation within the gene body they are in.

## Usage

```

relative_mutation_positions(
  mutations,
  annotation,
  bin = FALSE,
  by = c(region = "gene_name"),
  threads = 1
)

```

## Arguments

mutations	either the tibble output from <code>find_variants</code> . It must have columns <code>seqnames</code> , <code>pos</code> , and a third column for specifying the gene id or gene name. The mutation must be within the gene region.
annotation	Either path to the annotation file (GTF/GFF) or a <code>GRanges</code> object of the gene annotation.
bin	<code>logical(1)</code> : whether to bin the relative positions into 100 bins.
by	<code>character(1)</code> : the column name in the annotation to match with the gene annotation. E.g. <code>c("region" = "gene_name")</code> to match the 'region' column in the mutations with the 'gene_name' column in the annotation.
threads	<code>integer(1)</code> : number of threads to use.

## Value

If `bin = FALSE`, a list of numeric vectors of relative positions of each mutation within the gene body. If `bin = TRUE`, a numeric vector of length 100 of the number of mutations in each bin.

**Examples**

```

outdir <- tempfile()
dir.create(outdir)
genome_fa <- system.file("extdata", "rps24.fa.gz", package = "FLAMES")
minimap2_align( # align to genome
  config = jsonlite::fromJSON(
    system.file("extdata", "config_sclr_nanopore_3end.json", package = "FLAMES")),
  fa_file = genome_fa,
  fq_in = system.file("extdata", "fastq", "demultiplexed.fq.gz", package = "FLAMES"),
  annot = system.file("extdata", "rps24.gtf.gz", package = "FLAMES"),
  outdir = outdir
)
variants <- find_variants(
  bam_path = file.path(outdir, "align2genome.bam"),
  reference = genome_fa,
  annotation = system.file("extdata", "rps24.gtf.gz", package = "FLAMES"),
  min_nucleotide_depth = 4
)
positions <-
  relative_mutation_positions(
    mutations = variants,
    annotation = system.file("extdata", "rps24.gtf.gz", package = "FLAMES")
  )

```

---

relative\_mutation\_positions\_single

*Relative mutation positions within the gene body*


---

**Description**

Given a set of mutations and a gene annotation, calculate the relative position of each mutation within the gene body. The gene annotation must have the following types: "gene" and "exon". The gene annotation must be for one gene only. The mutations must be within the gene region. The function will merge overlapping exons and calculate the relative position of each mutation within the gene body, excluding intronic regions.

**Usage**

```

relative_mutation_positions_single(
  mutations,
  annotation_grange,
  verbose = TRUE
)

```

**Arguments**

mutations	either the tibble output from <code>find_variants</code> or a <code>GRanges</code> object. Make sure to filter it for only the gene of interest.
annotation_grange	<code>GRanges</code> : the gene annotation. Must have the following types: "gene" and "exon".
verbose	logical(1): whether to print messages.

**Value**

A numeric vector of relative positions of each mutation within the gene body. Ranging from 0 (start of the gene) to 1 (end of the gene).

---

scmixology\_lib10      *scMixology short-read gene counts - sample 2*

---

**Description**

Short-read gene counts from long and short-read single cell RNA-seq profiling of human lung adenocarcinoma cell lines using 10X version 2 chemistry. See Tian, L. et al. Comprehensive characterization of single-cell full-length isoforms in human and mouse with long-read sequencing. *Genome Biology* 22, 310 (2021).

**Usage**

```
scmixology_lib10
```

**Format**

```
## 'scmixology_lib10' A SingleCellExperiment with 7,240 rows and 60 columns:
```

**Source**

```
<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154869>
```

---

scmixology\_lib10\_transcripts      *scMixology long-read transcript counts - sample 2*

---

**Description**

long-read transcript counts from long and short-read single cell RNA-seq profiling of human lung adenocarcinoma cell lines using 10X version 2 chemistry. See Tian, L. et al. Comprehensive characterization of single-cell full-length isoforms in human and mouse with long-read sequencing. *Genome Biology* 22, 310 (2021).

**Usage**

```
scmixology_lib10_transcripts
```

**Format**

```
## 'scmixology_lib10_transcripts' A SingleCellExperiment with 7,240 rows and 60 columns:
```

**Source**

```
<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154869>
```

---

scmixology\_lib90      *scMixology short-read gene counts - sample 1*

---

### Description

Short-read single cell RNA-seq profiling of human lung adenocarcinoma cell lines using 10X version 2 chemistry. Single cells from five human lung adenocarcinoma cell lines (H2228, H1975, A549, H838 and HCC827) were mixed in equal proportions and processed using the Chromium 10X platform, then sequenced using Illumina HiSeq 2500. See Tian L, Dong X, Freytag S, Lê Cao KA et al. Benchmarking single cell RNA-sequencing analysis pipelines using mixture control experiments. Nat Methods 2019 Jun;16(6):479-487. PMID: 31133762

### Usage

```
scmixology_lib90
```

### Format

```
## 'scmixology_lib90' A SingleCellExperiment
```

### Source

```
<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE126906>
```

---

sc\_DTU\_analysis      *FLAMES Differential Transcript Usage Analysis*

---

### Description

Chi-square based differential transcription usage analysis. This variant is meant for single cell data. Takes the `SingleCellExperiment` object from `sc_long_pipeline` as input. Alternatively, the path to the output folder could be provided instead of the SCE object.

### Usage

```
sc_DTU_analysis(sce, min_count = 15)
```

### Arguments

<code>sce</code>	The <code>SingleCellExperiment</code> object from <code>sc_long_pipeline</code> , with the following metadata: <code>file</code> is required under the output folder of the SCE object.
<code>min_count</code>	The minimum UMI count threshold for filtering isoforms.

### Details

This function will search for genes that have at least two isoforms, each with more than `min_count` UMI counts. For each gene, the per cell transcript counts were merged by group to generate pseudo bulk samples. Grouping is specified by the `colLabels` of the SCE object. The top 2 highly expressed transcripts for each group were selected and a UMI count matrix where the rows are selected transcripts and columns are groups was used as input to a chi-square test of independence (`chisq.test`). Adjusted P-values were calculated by Benjamini–Hochberg correction.





**Arguments**

combined_sce	A SingleCellExperiment object with gene counts and a "transcript" altExp slot.
dimred	The name of the reduced dimension to use for building the shared nearest neighbor graph.
...	Additional arguments to pass to <code>scran::buildSNNGraph</code> . E.g. <code>k = 30</code> .

**Details**

For cells with NA values in the "transcript" altExp slot, this function imputes the missing values from cells with non-missing values. A shared nearest neighbor graph is built using reduced dimensions from the SingleCellExperiment object, and the imputation is done where the imputed value for a cell is the weighted sum of the transcript counts of its neighbors. Imputed values are stored in the "logcounts" assay of the "transcript" altExp slot. The "counts" assay is used to obtain logcounts but left unchanged.

**Value**

A SingleCellExperiment object with imputed logcounts assay in the "transcript" altExp slot.

**Examples**

```
sce <- SingleCellExperiment::SingleCellExperiment(assays = list(counts = matrix(rpois(50, 5), ncol = 10)))
long_read <- SingleCellExperiment::SingleCellExperiment(assays = list(counts = matrix(rpois(40, 5), ncol = 10)))
SingleCellExperiment::altExp(sce, "transcript") <- long_read
SingleCellExperiment::counts(SingleCellExperiment::altExp(sce))[,1:2] <- NA
SingleCellExperiment::counts(SingleCellExperiment::altExp(sce))
imputed_sce <- sc_impute_transcript(sce, k = 4)
SingleCellExperiment::logcounts(SingleCellExperiment::altExp(imputed_sce))
```

---

sc\_long\_multisample\_pipeline

*Pipeline for Multi-sample Single Cell Data*

---

**Description**

Semi-supervised isoform detection and annotation for long read data. This variant is for multi-sample single cell data. By default, this pipeline demultiplexes input fastq data (`match_cell_barcode = TRUE`). Specific parameters relating to analysis can be changed either through function arguments, or through a configuration JSON file.

**Usage**

```
sc_long_multisample_pipeline(
  annotation,
  fastqs,
  outdir,
  genome_fa,
  minimap2 = NULL,
  k8 = NULL,
```

```

    barcodes_file = NULL,
    expect_cell_numbers = NULL,
    config_file = NULL
)

```

### Arguments

annotation	The file path to the annotation file in GFF3 format
fastqs	The input fastq files for multiple samples. Should be a named vector of file paths (either to FASTQ files or directories containing FASTQ files). The names of the vector will be used as the sample names.
outdir	The path to directory to store all output files.
genome_fa	The file path to genome fasta file.
minimap2	Path to minimap2, if it is not in PATH. Only required if either or both of do_genome_align and do_read_realign are TRUE.
k8	Path to the k8 Javascript shell binary. Only required if do_genome_align is TRUE.
barcodes_file	The file path to the reference csv used for demultiplexing in flexplex. If not specified, the demultiplexing will be performed using BLAZE. Default is NULL.
expect_cell_numbers	A vector of roughly expected numbers of cells in each sample E.g., the targeted number of cells. Required if using BLAZE for demultiplexing, specifically, when the do_barcode_demultiplex are TRUE in the the JSON configuration file and barcodes_file is not specified. Default is NULL.
config_file	File path to the JSON configuration file. If specified, config_file overrides all configuration parameters

### Details

By default FLAMES use minimap2 for read alignment. After the genome alignment step (do\_genome\_align), FLAMES summarizes the alignment for each read in every sample by grouping reads with similar splice junctions to get a raw isoform annotation (do\_isoform\_id). The raw isoform annotation is compared against the reference annotation to correct potential splice site and transcript start/end errors. Transcripts that have similar splice junctions and transcript start/end to the reference transcript are merged with the reference. This process will also collapse isoforms that are likely to be truncated transcripts. If isoform\_id\_bambu is set to TRUE, bambu : bambu will be used to generate the updated annotations (Not implemented for multi-sample yet). Next is the read realignment step (do\_read\_realign), where the sequence of each transcript from the update annotation is extracted, and the reads are realigned to this updated transcript\_assembly.fa by minimap2. The transcripts with only a few full-length aligned reads are discarded (Not implemented for multi-sample yet). The reads are assigned to transcripts based on both alignment score, fractions of reads aligned and transcript coverage. Reads that cannot be uniquely assigned to transcripts or have low transcript coverage are discarded. The UMI transcript count matrix is generated by collapsing the reads with the same UMI in a similar way to what is done for short-read scRNA-seq data, but allowing for an edit distance of up to 2 by default. Most of the parameters, such as the minimal distance to splice site and minimal percentage of transcript coverage can be modified by the JSON configuration file (config\_file).

The default parameters can be changed either through the function arguments or through the configuration JSON file config\_file. the pipeline\_parameters section specifies which steps are to be executed in the pipeline - by default, all steps are executed. The isoform\_parameters section affects isoform detection - key parameters include:

Min\_sup\_cnt which causes transcripts with less reads aligned than it's value to be discarded  
 MAX\_TS\_DIST which merges transcripts with the same intron chain and TSS/TES distance less than  
 MAX\_TS\_DIST  
 strand\_specific which specifies if reads are in the same strand as the mRNA (1), or the reverse  
 complemented (-1) or not strand specific (0), which results in strand information being based  
 on reference annotation.

## Value

If "do\_transcript\_quantification" set to true, a list with two elements:

**metadata** A list of metadata from the pipeline run.

**sces** A list of SingleCellExperiment objects, one for each sample.

## See Also

[bulk\\_long\\_pipeline\(\)](#) for bulk long data, [SingleCellExperiment\(\)](#) for how data is outputted

## Examples

```
reads <- ShortRead::readFastq(
  system.file("extdata", "fastq", "muscrps24.fastq.gz", package = "FLAMES")
)
outdir <- tempfile()
dir.create(outdir)
dir.create(file.path(outdir, "fastq"))
bc_allow <- file.path(outdir, "bc_allow.tsv")
genome_fa <- file.path(outdir, "rps24.fa")
R.utils::gunzip(
  filename = system.file("extdata", "bc_allow.tsv.gz", package = "FLAMES"),
  destname = bc_allow, remove = FALSE
)
R.utils::gunzip(
  filename = system.file("extdata", "rps24.fa.gz", package = "FLAMES"),
  destname = genome_fa, remove = FALSE
)
ShortRead::writeFastq(reads[1:100],
  file.path(outdir, "fastq/sample1.fq.gz"), mode = "w", full = FALSE)
reads <- reads[-(1:100)]
ShortRead::writeFastq(reads[1:100],
  file.path(outdir, "fastq/sample2.fq.gz"), mode = "w", full = FALSE)
reads <- reads[-(1:100)]
ShortRead::writeFastq(reads,
  file.path(outdir, "fastq/sample3.fq.gz"), mode = "w", full = FALSE)

sce_list <- FLAMES::sc_long_multisample_pipeline(
  annotation = system.file("extdata", "rps24.gtf.gz", package = "FLAMES"),
  fastqs = c("sampleA" = file.path(outdir, "fastq"),
    "sample1" = file.path(outdir, "fastq", "sample1.fq.gz"),
    "sample2" = file.path(outdir, "fastq", "sample2.fq.gz"),
    "sample3" = file.path(outdir, "fastq", "sample3.fq.gz")),
  outdir = outdir,
  genome_fa = genome_fa,
  barcodes_file = rep(bc_allow, 4)
)
```

**Description**

Semi-supervised isoform detection and annotation for long read data. This variant is for single cell data. By default, this pipeline demultiplexes input fastq data (`match_cell_barcode = TRUE`). Specific parameters relating to analysis can be changed either through function arguments, or through a configuration JSON file.

**Usage**

```
sc_long_pipeline(
  annotation,
  fastq,
  genome_bam = NULL,
  outdir,
  genome_fa,
  minimap2 = NULL,
  k8 = NULL,
  barcodes_file = NULL,
  expect_cell_number = NULL,
  config_file = NULL
)
```

**Arguments**

<code>annotation</code>	The file path to the annotation file in GFF3 format
<code>fastq</code>	The file path to input fastq file
<code>genome_bam</code>	Optional file path to a bam file to use instead of fastq file (skips initial alignment step)
<code>outdir</code>	The path to directory to store all output files.
<code>genome_fa</code>	The file path to genome fasta file.
<code>minimap2</code>	Path to minimap2, if it is not in PATH. Only required if either or both of <code>do_genome_align</code> and <code>do_read_realign</code> are TRUE.
<code>k8</code>	Path to the k8 Javascript shell binary. Only required if <code>do_genome_align</code> is TRUE.
<code>barcodes_file</code>	The file path to the reference csv used for demultiplexing in flexiplex. If not specified, the demultiplexing will be performed using BLAZE. Default is NULL.
<code>expect_cell_number</code>	Expected number of cells for identifying the barcode list in BLAZE. This could be just a rough estimate. E.g., the targeted number of cells. Required if the <code>do_barcode_demultiplex</code> are TRUE in the the JSON configuration file and <code>barcodes_file</code> is not specified. Default is NULL.
<code>config_file</code>	File path to the JSON configuration file. If specified, <code>config_file</code> overrides all configuration parameters

## Details

By default FLAMES use minimap2 for read alignment. After the genome alignment step (`do_genome_align`), FLAMES summarizes the alignment for each read by grouping reads with similar splice junctions to get a raw isoform annotation (`do_isoform_id`). The raw isoform annotation is compared against the reference annotation to correct potential splice site and transcript start/end errors. Transcripts that have similar splice junctions and transcript start/end to the reference transcript are merged with the reference. This process will also collapse isoforms that are likely to be truncated transcripts. If `isoform_id_bambu` is set to TRUE, `bambu::bambu` will be used to generate the updated annotations. Next is the read realignment step (`do_read_realign`), where the sequence of each transcript from the update annotation is extracted, and the reads are realigned to this updated `transcript_assembly.fa` by minimap2. The transcripts with only a few full-length aligned reads are discarded. The reads are assigned to transcripts based on both alignment score, fractions of reads aligned and transcript coverage. Reads that cannot be uniquely assigned to transcripts or have low transcript coverage are discarded. The UMI transcript count matrix is generated by collapsing the reads with the same UMI in a similar way to what is done for short-read scRNA-seq data, but allowing for an edit distance of up to 2 by default. Most of the parameters, such as the minimal distance to splice site and minimal percentage of transcript coverage can be modified by the JSON configuration file (`config_file`).

The default parameters can be changed either through the function arguments or through the configuration JSON file `config_file`. the `pipeline_parameters` section specifies which steps are to be executed in the pipeline - by default, all steps are executed. The `isoform_parameters` section affects isoform detection - key parameters include:

`Min_sup_cnt` which causes transcripts with less reads aligned than it's value to be discarded

`MAX_TS_DIST` which merges transcripts with the same intron chain and TSS/TES distace less than `MAX_TS_DIST`

`strand_specific` which specifies if reads are in the same strand as the mRNA (1), or the reverse complemented (-1) or not strand specific (0), which results in strand information being based on reference annotation.

## Value

if `do_transcript_quantification` set to true, `sc_long_pipeline` returns a `SingleCellExperiment` object, containing a count matrix as an assay, gene annotations under metadata, as well as a list of the other output files generated by the pipeline. The pipeline also outputs a number of output files into the given `outdir` directory. These output files generated by the pipeline are:

**transcript\_count.csv.gz** - a transcript count matrix (also contained in the `SingleCellExperiment`)

**isoform\_annotated.filtered.gff3** - isoforms in gff3 format (also contained in the `SingleCellExperiment`)

**transcript\_assembly.fa** - transcript sequence from the isoforms

**align2genome.bam** - sorted BAM file with reads aligned to genome

**realign2transcript.bam** - sorted realigned BAM file using the `transcript_assembly.fa` as reference

**tss\_tes.bedgraph** - TSS TES enrichment for all reads (for QC)

if `do_transcript_quantification` set to false, nothing will be returned

## See Also

[bulk\\_long\\_pipeline\(\)](#) for bulk long data, [SingleCellExperiment\(\)](#) for how data is outputted

**Examples**

```

outdir <- tempfile()
dir.create(outdir)
bc_allow <- file.path(outdir, "bc_allow.tsv")
genome_fa <- file.path(outdir, "rps24.fa")
R.utils::gunzip(
  filename = system.file("extdata", "bc_allow.tsv.gz", package = "FLAMES"),
  destname = bc_allow, remove = FALSE
)
R.utils::gunzip(
  filename = system.file("extdata", "rps24.fa.gz", package = "FLAMES"),
  destname = genome_fa, remove = FALSE
)
if (!any(is.na(find_bin(c("minimap2", "k8"))))) {
  sce <- FLAMES::sc_long_pipeline(
    genome_fa = genome_fa,
    fastq = system.file("extdata", "fastq", "muscle_rps24.fastq.gz", package = "FLAMES"),
    annotation = system.file("extdata", "rps24.gtf.gz", package = "FLAMES"),
    outdir = outdir,
    barcodes_file = bc_allow
  )
}

```

---

sc\_mutations

*Variant count for single-cell data*


---

**Description**

Count the number of reads supporting each variants at the given positions for each cell.

**Usage**

```

sc_mutations(
  bam_path,
  seqnames,
  positions,
  indel = FALSE,
  barcodes,
  threads = 1
)

```

**Arguments**

bam_path	character(1) or character(n): path to the bam file(s) aligned to the reference genome (NOT the transcriptome! Unless the positions are also from the transcriptome).
seqnames	character(n): chromosome names of the positions to count alleles.
positions	integer(n): positions, 1-based, same length as seqnames. The positions to count alleles.
indel	logical(1): whether to count indels (TRUE) or SNPs (FALSE).

barcodes	character(n) when bam_path is a single file, or list of character(n) when bam_path is a list of files paths. The cell barcodes to count alleles for. Only reads with these barcodes will be counted.
threads	integer(1): number of threads to use. Maximum number of threads is the number of bam files * number of positions.

### Value

A tibble with columns: allele, barcode, allele\_count, cell\_total\_reads, pct, pos, seqname.

### Examples

```

outdir <- tempfile()
dir.create(outdir)
genome_fa <- file.path(outdir, "rps24.fa")
R.utils::gunzip(
  filename = system.file("extdata", "rps24.fa.gz", package = "FLAMES"),
  destname = genome_fa, remove = FALSE
)
minimap2_align( # align to genome
  config = jsonlite::fromJSON(
    system.file("extdata", "config_sclr_nanopore_3end.json", package = "FLAMES")
  ),
  fa_file = genome_fa,
  fq_in = system.file("extdata", "fastq", "demultiplexed.fq.gz", package = "FLAMES"),
  annot = system.file("extdata", "rps24.gtf.gz", package = "FLAMES"),
  outdir = outdir
)
snps_tb <- sc_mutations(
  bam_path = file.path(outdir, "align2genome.bam"),
  seqnames = c("chr14", "chr14"),
  positions = c(1260, 2714), # positions of interest
  indel = FALSE,
  barcodes = read.delim(
    system.file("extdata", "bc_allow.tsv.gz", package = "FLAMES"),
    header = FALSE)$V1
)
head(snps_tb)
snps_tb |>
  dplyr::filter(pos == 1260) |>
  dplyr::group_by(allele) |>
  dplyr::summarise(count = sum(allele_count)) # should be identical to samtools pileup

```

---

weight\_transcripts      *Weight transcripts by read counts*

---

### Description

Given a vector of read counts, return a vector of weights. The weights could be either the read counts themselves (type = 'counts'), a binary vector of 0s and 1s where 1s are assigned to transcripts with read counts above a threshold (type = 'equal', min\_counts = 1000), or a sigmoid function of the read counts (type = 'sigmoid'). The sigmoid function is defined as  $1 / (1 + \exp(-\text{steepness}/\text{inflection} * (x - \text{inflection})))$ .



**Usage**

```
weight_transcripts(  
  counts,  
  type = "sigmoid",  
  min_counts = 1000,  
  inflection_idx = 10,  
  inflection_max = 1000,  
  steepness = 5  
)
```

**Arguments**

counts	numeric vector of read counts
type	string, one of 'counts', 'sigmoid', or 'equal'
min_counts	numeric, the threshold for the 'equal' type
inflection_idx	numeric, the index of the read counts to determine the inflection point for the sigmoid function. The default is 10, i.e. the 10th highest read count will be the inflection point.
inflection_max	numeric, the maximum value for the inflection point. If the inflection point according to the inflection_idx is higher than this value, the inflection point will be set to this value instead.
steepness	numeric, the steepness of the sigmoid function

**Value**

numeric vector of weights

**Examples**

```
weight_transcripts(1:2000)  
par(mfrow = c(2, 2))  
plot(  
  1:2000, weight_transcripts(1:2000, type = 'sigmoid'),  
  type = 'l', xlab = 'Read counts', ylab = 'Sigmoid weight'  
)  
plot(  
  1:2000, weight_transcripts(1:2000, type = 'counts'),  
  type = 'l', xlab = 'Read counts', ylab = 'Weight by counts'  
)  
plot(  
  1:2000, weight_transcripts(1:2000, type = 'equal'),  
  type = 'l', xlab = 'Read counts', ylab = 'Equal weights'  
)
```

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