

# Package ‘FLAMES’

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**Type** Package

**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

**License** GPL (>= 2)

**Encoding** UTF-8

**Imports** basilisk, reticulate, SingleCellExperiment, SummarizedExperiment, MultiAssayExperiment, Rsamtools, utils, zlibbioc, scater, dplyr, tidyr, magrittr, S4Vectors, scuttle, stats, rtracklayer, igraph, ggbio, GenomicRanges, Matrix, BiocGenerics, ggplot2, scran, ComplexHeatmap, RColorBrewer, circlize, grid, gridExtra, cowplot, stringr, bambu, GenomeInfoDb, withr, Biostrings, GenomicFeatures, jsonlite, parallel, DropletUtils

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---

annotation\_to\_fasta    *GTF/GFF to FASTA conversion*

---

### Description

convert the transcript annotation to transcriptome assembly as FASTA file.

### Usage

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

**Arguments**

isoform_annotation	Path to the annotation file (GTF/GFF3)
genome_fa	The file path to genome fasta file.
outdir	The path to directory to store the transcriptome as transcript_assembly.fa.

**Value**

Path to the outputted transcriptome assembly

**Examples**

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

---

bulk\_long\_pipeline      *Pipeline for Bulk Data*

---

**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_dir = 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_dir	Path to the directory containing minimap2, if it is not in PATH. Only required if either or both of do_genome_align and do_read_realign are 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 its 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-anno
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, "genome.fa", paste(file_url, "SIRV_isoforms_multi-fasta_17061
fastq_dir <- paste(temp_path, "fastq_dir", sep = "/") # the downloaded fastq files need to be in a directory to be me
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)
if (is.character(locate_minimap2_dir())) {
  se <- bulk_long_pipeline(
    annotation = annotation, fastq = fastq_dir, outdir = outdir, genome_fa = genome_fa,
    config_file = system.file("extdata/SIRV_config_default.json", package = "FLAMES")
  )

  se_2 <- create_se_from_dir(outdir = outdir, annotation = annotation)
}
```

---

callBasilisk

*Internal utility function for simplifying calls to basiliskRun using a given basilisk environment*

---

**Description**

Internal utility function for simplifying calls to basiliskRun using a given basilisk environment

**Usage**

```
callBasilisk(env_name, FUN, ...)
```

**Arguments**

env_name	the name of the basilisk env (made through BasiliskEnvironment) to execute code within
FUN	the function to execute from with the basilisk environment
...	extra parameters required by FUN

**Value**

the result of 'FUN'

---

combine\_sce

*Combine SCE*

---

**Description**

Combine long- and short-read SingleCellExperiment objects

**Usage**

```
combine_sce(
  short_read_large,
  short_read_small,
  long_read_sce,
  remove_duplicates = FALSE
)
```

**Arguments**

short\_read\_large

The SCE object, or path to the HDF5 file, or folder containing the matrix file, corresponding to the larger short-read sample

short\_read\_small

The SCE object, or path to the HDF5 file, or folder containing the matrix file, corresponding to the smaller short-read sample

long\_read\_sce The SCE object of the transcript counts, from the long-read pipelines.

remove\_duplicates

determines whether cells with duplicated barcodes are kept in the smaller library ( they are always removed from the larger library)

**Details**

Takes the long-read SCE object from the long-read pipeline and the short-read SCE object, creates a MultiAssayExperiment object with the two SingleCellExperiment objects. Cells with duplicated barcodes are removed from the larger library.

**Value**

A MultiAssayExperiment object, with "gene\_counts" and "transcript\_counts" experiments.

**Examples**

```

library(SingleCellExperiment)
a <- SingleCellExperiment(assays = list(counts = matrix(rpois(100, 5), ncol = 10)))
b <- SingleCellExperiment(assays = list(counts = matrix(rpois(100, 5), ncol = 10)))
long_read <- SingleCellExperiment(assays = list(counts = matrix(rpois(100, 5), ncol = 10)))
colData(a)$Barcode <- paste0(1:10, "-1")
colData(b)$Barcode <- paste0(8:17, "-1")
colnames(long_read) <- as.character(2:11)
rownames(a) <- as.character(101:110)
rownames(b) <- as.character(103:112)
rownames(long_read) <- as.character(1001:1010)
combine_sce(short_read_large = a, short_read_small = b, long_read_sce = long_read)

```

create\_config

*Create Configuration File From Arguments***Description**

Create Configuration File From Arguments

**Usage**

```
create_config(outdir, type = "sc_5end", ...)
```

**Arguments**

outdir	the destination directory for the configuration nfile
type	use an example config, available values: <ul style="list-style-type: none"> <li>• "sc_5end" - config for 5' end ONT reads</li> <li>• "SIRV" - config for the SIRV example reads</li> </ul>
...	Configuration parameters. <ul style="list-style-type: none"> <li>• do_genome_align - Boolean. Specifies whether to run the genome alignment step. TRUE is recommended</li> <li>• do_isoform_id - Boolean. Specifies whether to run the isoform identification step. TRUE is recommended</li> <li>• do_read_realign - Boolean. Specifies whether to run the read realignment step. TRUE is recommended</li> <li>• do_transcript_quant - Boolean. Specifies whether to run the transcript quantification step. TRUE is recommended</li> <li>• gen_raw_isoform - Boolean.</li> <li>• has_UMI - Boolean. Specifies if the data contains UMI.</li> <li>• max_dist - Maximum distance allowed when merging splicing sites in isoform consensus clustering.</li> </ul>

- max\_ts\_dist - Maximum distance allowed when merging transcript start/end position in isoform consensus clustering.
- max\_splice\_match\_dist - Maximum distance allowed when merging splice site called from the data and the reference annotation.
- min\_fl\_exon\_len - Minimum length for the first exon outside the gene body in reference annotation. This is to correct the alignment artifact
- max\_site\_per\_splice - Maximum transcript start/end site combinations allowed per splice chain
- min\_sup\_cnt - Minimum number of read support an isoform decrease this number will significantly increase the number of isoform detected.
- min\_cnt\_pct - Minimum percentage of count for an isoform relative to total count for the same gene.
- min\_sup\_pct - Minimum percentage of count for a splice chain that support a given transcript start/end site combination.
- strand\_specific - 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 strengte 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 Minimap2 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\_5end.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 = TRUE)
R.utils::gunzip(filename = system.file("extdata/rps24.fa.gz", package = "FLAMES"), destname = genome_fa, remove = TRUE)
annotation <- system.file("extdata/rps24.gtf.gz", package = "FLAMES")

if (is.character(locate_minimap2_dir())) {
  sce <- FLAMES::sc_long_pipeline(
    genome_fa = genome_fa,
    fastq = system.file("extdata/fastq", package = "FLAMES"),
    annotation = annotation,
    outdir = outdir,
    match_barcode = TRUE,
    reference_csv = bc_allow
  )
  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-anno
genome_fa <- bfc[[names(BiocFileCache::bfcadd(bfc, "genome.fa", paste(file_url, "SIRV_isoforms_multi-fasta_17061
fastq_dir <- paste(temp_path, "fastq_dir", sep = "/") # the downloaded fastq files need to be in a directory to be me
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)
if (is.character(locate_minimap2_dir())) {
  se <- bulk_long_pipeline(
    annotation = annotation, fastq = fastq_dir, outdir = outdir, genome_fa = genome_fa,
    config_file = system.file("extdata/SIRV_config_default.json", package = "FLAMES")
  )

  se_2 <- create_se_from_dir(outdir = outdir, annotation = annotation)
}
```

---

demultiplex\_sockeye     *Demultiplex reads using Sockeye outputs*

---

### Description

Demultiplex reads using the `cell_umi_gene.tsv` file from Sockeye.

### Usage

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

### Arguments

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

### Value

returns NULL

---

`find_barcode`     *Match Cell Barcodes*

---

### Description

Match cell barcodes in the given fastq directory with the reference csv, `ref_csv`. Matches are returned in the output file `out_fastq`. The flanking sequence is aligned to the first 30000 reads to identify the regions where cell barcode is likely to be found within. Next, sequences within this region are matched to barcodes in `ref_csv`, allowing `MAX_DIST` hamming distances. Reads that are successfully matched with a barcode are reported as the barcode `hm` match count. Every read that could not be matched in the previous step is aligned to the flanking sequence again to identify the location of barcode individually, and barcode matching is done with up to `MAX_DIST` levenshtein distances (allowing indels). Reads that are matched by this step is reported as the fuzzy match counts.

### Usage

```
find_barcode(
  fastq_dir,
  stats_file,
  out_fastq,
  ref_csv,
  MAX_DIST,
  UMI_LEN = 10L
)
```

**Arguments**

fastq_dir	directory containing fastq files to match
stats_file	NEEDED
out_fastq	output filename for matched barcodes
ref_csv	NEEDED
MAX_DIST	int; maximum edit distance
UMI_LEN	int; length of UMI sequences

**Value**

returns NULL

**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 = TRUE)
find_barcode(
  fastq_dir = system.file("extdata/fastq", package = "FLAMES"),
  stats_file = file.path(outdir, "bc_stat"),
  out_fastq = file.path(outdir, "demultiplexed.fq.gz"),
  ref_csv = bc_allow,
  MAX_DIST = 2,
  UMI_LEN = 10
)

```

---

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_17061
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta-anno
outdir <- tempfile()
dir.create(outdir)
if (is.character(locate_minimap2_dir())) {
  config <- jsonlite::fromJSON(system.file("extdata/SIRV_config_default.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
  )
}
```

---

get\_GRangesList

*Parse FLAMES' GFF output*


---

**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_17061
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta-anno
outdir <- tempfile()
dir.create(outdir)
if (is.character(locate_minimap2_dir())) {
  config <- jsonlite::fromJSON(system.file("extdata/SIRV_config_default.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"))
}

```

---

locate\_minimap2\_dir    *locate parent folder of minimap2*

---

**Description**

locate\_minimap2, or validate that minimap2 exists under minimap2\_dir

**Usage**

```
locate_minimap2_dir(minimap2_dir = NULL)
```

**Arguments**

minimap2\_dir    (Optional) folder that includes minimap2

**Value**

Path to the parent folder of minimap2 if available, or FALSE if minimap2 could not be found.

**Examples**

```
locate_minimap2_dir()
```

---

minimap2_align	<i>Minimap2 Align to Genome</i>
----------------	---------------------------------

---

### 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_dir,
    prefix = NULL,
    threads = NULL
)
```

### 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_dir	Path to the directory containing minimap2
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

Path to the alignment file

### 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_17061
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta-anno
outdir <- tempfile()
dir.create(outdir)
if (is.character(locate_minimap2_dir())) {
  minimap2_align(
    config = jsonlite::fromJSON(system.file("extdata/SIRV_config_default.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_dir,
  prefix = NULL,
  threads = NULL
)

```

**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_dir	Path to the directory containing minimap2
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**

Path to the alignment file

**See Also**

[minimap2\_align()]

**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_17061
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta-anno
outdir <- tempfile()
dir.create(outdir)
if (is.character(locate_minimap2_dir())) {
  fasta <- annotation_to_fasta(annotation, genome_fa, outdir)
  minimap2_realign(
    config = jsonlite::fromJSON(system.file("extdata/SIRV_config_default.json", package = "FLAMES")),
    fq_in = fastq1,
    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_1706
parsed_gff <- parse_gff_tree(gff)
```

---

 quantify

*Transcript quantification*


---

**Description**

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

**Usage**

```
quantify(annotation, outdir, config, pipeline = "sc_single_sample")
```

**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), <code>bulk</code> (bulk, single or multi-sample), or <code>sc_multi_sample</code> (single-cell, multiple samples)

**Value**

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

**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_17061
annotation <- bfc[[names(BiocFileCache::bfcadd(bfc, "annot.gtf", paste(file_url, "SIRV_isoforms_multi-fasta-anno
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, mi
file.copy(annotation, file.path(outdir, "isoform_annotated.gtf"))
if (is.character(locate_minimap2_dir())) {
  minimap2_realign(
    config = config, outdir = outdir,
```

```

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

```

---

sc\_annotate\_plots      *FLAMES Annotated Plottings*

---

## Description

Plot isoform exons alignments for a given gene, along with UMAP showing expression levels.

## Usage

```

sc_annotate_plots(
  gene,
  multiAssay,
  cluster_annotation,
  n_isoforms = 4,
  n_pcs = 40,
  return_multiAssay = TRUE,
  heatmap_annotation_colors = "BrBG",
  isoform_legend_width = 7,
  heatmap_color_quantile = 0.95,
  col_low = "#313695",
  col_mid = "#FFFFBF",
  col_high = "#A50026"
)

```

## Arguments

gene	The gene symbol of interest.
multiAssay	The MultiAssayExperiment object from <code>combine_sce()</code> .
cluster_annotation	Path to the cluster annotation CSV (required for heatmap, if <code>cluster_annotation.csv</code> is not in path and <code>multiAssay\$cell_type</code> does not exist)
n_isoforms	The number of expressed isoforms to keep.
n_pcs	The number of principal components to generate.
return_multiAssay	Whether to return the processed MultiAssayExperiment object.
heatmap_annotation_colors	Name of color palette to use for cell group annotation in heatmaps, see <a href="#">RColorBrewer::brewer.pal()</a> available diverging palettes are: BrBG PiYG PRGn PuOr RdBu RdGy RdYlBu RdYlGn Spectral when there are more than 11 groups, this argument will be ignored and random palettes will be generated.

isoform_legend_width	The width of isoform legends in heatmaps, in cm.
heatmap_color_quantile	Float; Expression levels higher than this quantile will all be shown with col_high. Expression levels lower than 1 - heatmap_color_quantile will all be shown with col_low;
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.

### Details

This function takes a combined `MultiAssayExperiment` object containing "gene\_counts" and "transcript\_counts" experiments to generate a combined UMAP, the expression levels of isoforms (using long read data) are then overlaid on top of the UMAP. SNN inference based on gene counts were performed to impute isoform expression for cells in the larger library. The `MultiAssayExperiment` object should have a boolean column named "Lib\_small" in its column data file to indicate which subsample the cells are in. The `MultiAssayExperiment` object can be created using the `combine_sce` function.

### Value

a list containing the combined UMAP, the isoform exon alignments and the UMAP with isoform expression levels.

### See Also

[combine\\_sce\(\)](#) for combining gene count `SingleCellExperiment` object with transcript counts.

---

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. A cluster annotation file `cluster_annotation.csv` is required, please provide this file under the output folder of `sc_long_pipeline`.

### Usage

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

**Arguments**

sce	The SingleCellExperiment object from sc_long_pipeline, an additional cluster_annotation.csv file is required under the output folder of the SCE object.
path	The path to the output folder of sc_long_pipeline the folder needs to contain: <ul style="list-style-type: none"> <li>transcript_count.csv.gz - the transcript count matrix</li> <li>isoform_FSM_annotation.csv - the full splice match annotation file</li> <li>cluster_annotation.csv - cluster annotation file</li> </ul>
min_count	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 cluster\_annotation.csv file. 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.

**Value**

a data.frame containing the following columns:

- gene\_name - differentially transcribed genes
- X\_value - the X value for the DTU gene
- df - degrees of freedom of the approximate chi-squared distribution of the test statistic
- DTU\_tr - the transcript\_id with the highest squared residuals
- DTU\_group - the cell group with the highest squared residuals
- p\_value - the p-value for the test
- adj\_p - the adjusted p-value (by Benjamini–Hochberg correction)

The table is sorted by decreasing P-values. It will also be saved as sc\_DTU\_analysis.csv under the output folder.

**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 = TRUE)
R.utils::gunzip(filename = system.file("extdata/rps24.fa.gz", package = "FLAMES"), destname = genome_fa, remove = TRUE)

if (is.character(locate_minimap2_dir())) {
  sce <- FLAMES::sc_long_pipeline(
    genome_fa = genome_fa,
    fastq = system.file("extdata/fastq", package = "FLAMES"),
```

```

    annotation = system.file("extdata/rps24.gtf.gz", package = "FLAMES"),
    outdir = outdir,
    match_barcode = TRUE,
    reference_csv = bc_allow
  )
  group_anno <- data.frame(barcode_seq = colnames(sce), groups = SingleCellExperiment::counts(sce)["ENSMUST000001"]
  write.csv(group_anno, file.path(outdir, "cluster_annotation.csv"), row.names = FALSE)
  sc_DTU_analysis(sce, outdir, min_count = 1)
}

```

---

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_dir = NULL,
  reference_csv,
  match_barcode = TRUE,
  config_file = NULL
)

```

## Arguments

<code>annotation</code>	The file path to the annotation file in GFF3 format
<code>fastqs</code>	Paths to the folder containing fastq files, or vector of paths to each fastq file.
<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_dir</code>	Path to the directory containing 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>reference_csv</code>	The file path to the reference csv used for demultiplexing
<code>match_barcode</code>	Boolean; specifies if demultiplexing should be performed using <code>FLAMES::find_barcode</code>
<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 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 its 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

a list of `SingleCellExperiment` objects if "`do_transcript_quantification`" set to true. Otherwise nothing will be returned.

## See Also

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

## Examples

```
reads <- ShortRead::readFastq(system.file("extdata/fastq/musc_rps24.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, remov
```

```

R.utils::gunzip(filename = system.file("extdata/rps24.fa.gz", package = "FLAMES"), destname = genome_fa, remove =
ShortRead::writeFastq(sample(reads, size = 500, replace = TRUE), file.path(outdir, "fastq/sample1.fq.gz"), mode =
ShortRead::writeFastq(sample(reads, size = 500, replace = TRUE), file.path(outdir, "fastq/sample2.fq.gz"), mode =
ShortRead::writeFastq(sample(reads, size = 500, replace = TRUE), file.path(outdir, "fastq/sample3.fq.gz"), mode =

if (is.character(locate_minimap2_dir())) {
  sce_list <- FLAMES::sc_long_multisample_pipeline(
    annotation = system.file("extdata/rps24.gtf.gz", package = "FLAMES"),
    fastqs = file.path(outdir, "fastq", list.files(file.path(outdir, "fastq"))),
    outdir = outdir,
    genome_fa = genome_fa,
    reference_csv = rep(bc_allow, 3)
  )
}

```

---

sc\_long\_pipeline

*Pipeline for Single Cell Data*


---

## 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_dir = NULL,
  reference_csv,
  match_barcode,
  config_file = NULL
)

```

## Arguments

annotation	The file path to the annotation file in GFF3 format
fastq	The file path to input fastq file
genome_bam	Optional file path to a bam file to use instead of fastq file (skips initial alignment step)
outdir	The path to directory to store all output files.



genome_fa	The file path to genome fasta file.
minimap2_dir	Path to the directory containing minimap2, if it is not in PATH. Only required if either or both of do_genome_align and do_read_realign are TRUE.
reference_csv	The file path to the reference csv used for demultiplexing
match_barcode	Boolean; specifies if demultiplexing should be performed using FLAMES::find_barcode
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 its 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, 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 = TRUE)
R.utils::gunzip(filename = system.file("extdata/rps24.fa.gz", package = "FLAMES"), destname = genome_fa, remove = TRUE)

if (is.character(locate_minimap2_dir())) {
  sce <- FLAMES::sc_long_pipeline(
    genome_fa = genome_fa,
    fastq = system.file("extdata/fastq", package = "FLAMES"),
    annotation = system.file("extdata/rps24.gtf.gz", package = "FLAMES"),
    outdir = outdir,
    match_barcode = TRUE,
    reference_csv = bc_allow
  )
}
```

---

sc\_mutations

*FLAMES variant calling*

---

### Description

Candidate SNVs identified with filtering by coverage threshold, and allele frequency range.

### Usage

```
sc_mutations(
  sce,
  barcode_tsv,
  bam_short,
  out_dir,
```

```

    genome_fa,
    annot,
    known_positions = NULL,
    min_cov = 100,
    report_pct = c(0.1, 0.9)
)

```

### Arguments

sce	The SingleCellExperiment object from sc_long_pipeline
barcode_tsv	TSV file for cell barcodes
bam_short	(Optional) short read alignment BAM file. If provided, it is used to filter the variations. Variations in long-read data with enough short read coverage but no alternative allele will not be reported.
out_dir	(Optional) Output folder of sc_long_pipeline. Output files from this function will also be saved here. Use this parameter if you do not have the SingleCellExperiment object.
genome_fa	(Optional) Reference genome FASTA file. Use this parameter is if you do not wish sc_mutat ion to use the reference genome FASTA file from the sce's meta-data.
annot	(Optional) The file path to gene annotation file in gff3 format. If provided as FALSE then the isoform_annotated.gff3 from sc_longread_pipeline will be used, if not provided then the path in the SingleCellExperiment object will be used.
known_positions	(Optional) A list of known positions, with by chromosome name followed by the position, e.g. ('chr1', 123, 'chr1', 124, 'chrX', 567). These locations will not be filtered and its allele frequencies will be reported.
min_cov	The coverage threshold for filtering candidate SNVs. Positions with reads less than this number will not be considered.
report_pct	The allele frequency range for filtering candidate SNVs. Positions with less or higher allele frequency will not be reported. The default is 0.10-0.90

### Details

Takes the SingleCellExperiment object from sc\_long\_pipeline and the cell barcodes as barcode. Alternatively, input can also be provided as out\_dir, genome\_fa, annot, barcode.

### Value

a data.frame containing the following columns:

- chr - the chromosome where the mutation is located
- position
- REF - the reference allele
- ALT - the alternative allele

- REF\_frequency - reference allele frequency
- REF\_frequency\_in\_short\_reads - reference allele frequency in short reads (-1 when short reads not provided)
- hypergeom\_test\_p\_value
- sequence\_entropy
- INDEL\_frequency
- adj\_p - the adjusted p-value (by Benjamini–Hochberg correction)

The table is sorted by decreasing adjusted P value.

files saved to out\_dir/mutation:

- ref\_cnt.csv.gz
- alt\_cnt.csv.gz
- allele\_stat.csv.gz
- freq\_summary.csv

### 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 = TRUE)
R.utils::gunzip(filename = system.file("extdata/rps24.fa.gz", package = "FLAMES"), destname = genome_fa, remove = TRUE)

if (is.character(locate_minimap2_dir())) {
  sce <- FLAMES::sc_long_pipeline(
    genome_fa = genome_fa,
    fastq = system.file("extdata/fastq", package = "FLAMES"),
    annotation = system.file("extdata/rps24.gtf.gz", package = "FLAMES"),
    outdir = outdir,
    match_barcode = TRUE,
    reference_csv = bc_allow
  )
  sc_mutations(sce, barcode_tsv = file.path(outdir, "bc_allow.tsv"), min_cov = 2, report_pct = c(0, 1))
}

```

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