

# Package ‘Isosceles’

October 16, 2023

**Title** Isoform Single-Cell and Long-read Expression Suite

**Version** 0.0.2

**Description** Transcript detection and quantification from long reads.

**Depends** R (>= 4.2.0),  
SingleCellExperiment (>= 1.18.0)

**Imports** utils (>= 4.2.0),  
methods (>= 4.2.0),  
stats (>= 4.2.0),  
rlang (>= 1.0.4),  
assertthat (>= 0.2.1),  
magrittr (>= 2.0.3),  
tibble (>= 3.1.7),  
tidyselect (>= 1.1.2),  
dplyr (>= 1.0.9),  
tidyr (>= 1.2.0),  
glue (>= 1.6.2),  
digest (>= 0.6.29),  
Rcpp (>= 1.0.9),  
Matrix (>= 1.4-1),  
BiocParallel (>= 1.30.3),  
BiocNeighbors (>= 1.14.0),  
S4Vectors (>= 0.34.0),  
BiocGenerics (>= 0.42.0),  
Biostrings (>= 2.64.0),  
BSgenome (>= 1.64.0),  
GenomeInfoDb (>= 1.32.2),  
IRanges (>= 2.30.0),  
GenomicRanges (>= 1.48.0),  
Rsamtools (>= 2.12.0),  
GenomicAlignments (>= 1.32.1),  
rtracklayer (>= 1.56.1),  
GenomicFeatures (>= 1.48.3),  
SummarizedExperiment (>= 1.26.1),  
igraph (>= 1.3.4),  
scuttle (>= 1.6.2),

fastmatch ( $\geq$  1.1-3),  
pheatmap ( $\geq$  1.0.12),  
ggbio ( $\geq$  1.44.1),  
ggplot2 ( $\geq$  3.3.6),  
biovizBase ( $\geq$  1.44.0)

**License** GPL ( $\geq$  3)

**URL** <https://github.com/timbitz/Isosceles>

**Encoding** UTF-8

**Roxygen** list(markdown = TRUE)

**RoxygenNote** 7.2.1

**Suggests** testthat ( $\geq$  3.0.0),  
tools ( $\geq$  4.2.0),  
knitr ( $\geq$  1.39),  
rmarkdown ( $\geq$  2.14),  
BiocStyle ( $\geq$  2.24.0),  
viridis ( $\geq$  0.6.2),  
dittoSeq ( $\geq$  1.8.1),  
scraper ( $\geq$  1.24.0),  
scater ( $\geq$  1.24.0),  
bluster ( $\geq$  1.6.0)

**Config/testthat/edition** 3

**LinkingTo** Rcpp ( $\geq$  1.0.9),  
RcppArmadillo ( $\geq$  0.11.2.0.0)

**VignetteBuilder** knitr

R topics documented:

Isosceles-package . . . . .	3
bam_to_read_structures . . . . .	3
bam_to_tcc . . . . .	4
export_gtf . . . . .	5
neighborhood_tcc . . . . .	5
plot_psi_heatmap . . . . .	6
plot_psi_regions . . . . .	7
prepare_transcripts . . . . .	8
pseudobulk_tcc . . . . .	9
pseudotime_tcc . . . . .	9
tcc_to_gene . . . . .	10
tcc_to_transcript . . . . .	11
transcript_to_psi . . . . .	11
<b>Index</b>	<b>13</b>

---

Isosceles-package	<i>Isosceles: Isoform Single-Cell and Long-read Expression Suite</i>
-------------------	--

---

**Description**

Transcript detection and quantification from long reads

**Author(s)**

Tim Sterne-Weiler [sternewt@gene.com](mailto:sternewt@gene.com)

Michal Kabza [michal.kabza@contractors.roche.com](mailto:michal.kabza@contractors.roche.com)

---

`bam_to_read_structures`

*Extract read structures from BAM files*

---

**Description**

Extracts non-redundant read structures from one or multiple BAM files.

**Usage**

```
bam_to_read_structures(bam_files, chunk_size = 1e+06, ncpu = 1)
```

**Arguments**

<code>bam_files</code>	A character vector containing BAM file paths.
<code>chunk_size</code>	An integer scalar specifying the chunk size for reading the BAM files.
<code>ncpu</code>	An integer scalar specifying the number of cores to use for multicore parallelization.

**Value**

A data frame containing non-redundant read structure data obtained from the BAM files.

bam\_to\_tcc

*Prepare a TCC SummarizedExperiment object***Description**

Prepares a TCC (Transcript Compatibility Counts) SummarizedExperiment object for the given BAM files and transcript set.

**Usage**

```
bam_to_tcc(
  bam_files,
  transcript_data,
  run_mode = "strict",
  min_read_count = 1,
  min_relative_expression = 0.1,
  extend_spliced_transcripts = 100,
  is_single_cell = FALSE,
  barcode_tag = "BC",
  chunk_size = 1e+06,
  ncpu = 1
)
```

**Arguments**

bam_files	A named character vector containing BAM file paths.
transcript_data	A named list containing transcript data returned by the <a href="#">prepare_transcripts</a> function.
run_mode	A string specifying the mode for choosing the transcript set ('strict', 'de_novo_strict', 'de_novo_loose' or 'de_novo_full').
min_read_count	An integer scalar specifying the read count threshold for transcripts extracted from the BAM files.
min_relative_expression	A numeric scalar specifying the relative expression threshold for transcripts extracted from the BAM files.
extend_spliced_transcripts	An integer scalar specifying the number of base pairs by which transcript starts and ends are extended for spliced read compatibility search.
is_single_cell	A logical scalar specifying if the BAM files contain single cell data.
barcode_tag	A string specifying the name of the BAM file tag containing cell barcodes.
chunk_size	An integer scalar specifying the chunk size for reading the BAM files.
ncpu	An integer scalar specifying the number of cores to use for multicore parallelization.

**Value**

A SummarizedExperiment object containing TCC annotation and quantification data.

---

export_gtf	<i>Export data to a GTF file</i>
------------	----------------------------------

---

**Description**

Exports transcripts from a SummarizedExperiment to a GTF file.

**Usage**

```
export_gtf(se, file)
```

**Arguments**

se	A transcript-level SummarizedExperiment object returned by the <a href="#">tcc_to_transcript</a> function.
file	A string specifying the output file path.

**Value**

Nothing is returned.

---

neighborhood_tcc	<i>Merge the neighboring cell TCC values in scRNA-Seq data</i>
------------------	--

---

**Description**

Prepares a TCC SummarizedExperiment object where count values from the nearest k neighbors are added to the count values of each cell.

**Usage**

```
neighborhood_tcc(se_tcc, pca_mat, k = 10, use_annoy = FALSE, ncpu = 1)
```

**Arguments**

se_tcc	A TCC SummarizedExperiment object returned by the <a href="#">bam_to_tcc</a> function.
pca_mat	A matrix containing PCA coordinates of each cell.
k	An integer scalar specifying the number of nearest neighbors to use.
use_annoy	A logical scalar indicating whether to use the Annoy algorithm for approximate nearest neighbor identification (recommended for big datasets).
ncpu	An integer scalar specifying the number of cores to use for multicore parallelization.

**Value**

A SummarizedExperiment object containing merged TCC data.

---

plot_psi_heatmap	<i>Plot a PSI heatmap</i>
------------------	---------------------------

---

**Description**

Creates a heatmap of PSI (Percent Spliced In) values for the regions of a given gene across samples or cells.

**Usage**

```
plot_psi_heatmap(  
  se_psi,  
  gene_id,  
  heatmap_colors = viridis::cividis(100),  
  region_colors = NULL,  
  ...  
)
```

**Arguments**

se_psi	A PSI SummarizedExperiment object returned by the <a href="#">transcript_to_psi</a> function.
gene_id	A string containing the identifier of the gene to plot.
heatmap_colors	A character vector containing the color palette used in the heatmap.
region_colors	A named character vector of colors for the region type annotations.
...	Additional parameters for the plot, passed to the <a href="#">pheatmap</a> function.

**Value**

A plot object.

---

plot_psi_regions	<i>Plot PSI regions</i>
------------------	-------------------------

---

## Description

Creates a plot showing PSI regions and transcript structures for the given gene. Individual transcript structures are colored by their relative expression, calculated from the overall TPM values and expressed in percentages. For better visualization, introns can be shrunk using the `max_intron_length` argument.

## Usage

```
plot_psi_regions(  
  se_psi,  
  se_transcript,  
  gene_id,  
  max_transcripts = Inf,  
  max_intron_length = NULL,  
  region_colors = NULL  
)
```

## Arguments

<code>se_psi</code>	A PSI SummarizedExperiment object returned by the <a href="#">transcript_to_psi</a> function.
<code>se_transcript</code>	A transcript-level SummarizedExperiment object returned by the <a href="#">tcc_to_transcript</a> function.
<code>gene_id</code>	A string containing the identifier of the gene to plot.
<code>max_transcripts</code>	An integer scalar specifying the maximum number of transcripts with the highest relative expression to plot.
<code>max_intron_length</code>	An integer scalar specifying the maximum intron length after shrinking. If set to NULL, no shrinking is performed.
<code>region_colors</code>	A named character vector of colors for the PSI region types.

## Value

A plot object.

---

```
prepare_transcripts    Prepare transcript data for the analysis
```

---

## Description

Prepares transcript data (reference and extracted from the BAM files) for further analysis.

## Usage

```
prepare_transcripts(
  gtf_file,
  genome_fasta_file,
  bam_parsed,
  min_intron_length = 30,
  known_intron_motifs = c("GT-AG"),
  rescue_annotated_introns = FALSE,
  known_intron_granges = NULL,
  min_bam_splice_read_count = 2,
  min_bam_splice_fraction = 0.1,
  bin_size = 50
)
```

## Arguments

gtf_file	A string containing a GTF file path.
genome_fasta_file	A string containing a genome FASTA file path.
bam_parsed	A data frame containing non-redundant read structure data returned by the <a href="#">bam_to_read_structures</a> function. If NULL, only reference transcripts are used.
min_intron_length	An integer scalar specifying the minimal length of introns to assign strand to.
known_intron_motifs	A character vector specifying the known intron motifs.
rescue_annotated_introns	A logical scalar specifying if introns found in genome annotations should be kept even if they don't have known intron motifs.
known_intron_granges	A GRanges object storing known intron positions (e.g. from short read data) used for transcript classification. If set to NULL, only introns from reference annotations are used.
min_bam_splice_read_count	An integer scalar specifying the read count threshold for splice sites confirmed by aligned reads.
min_bam_splice_fraction	A numeric scalar specifying the minimum connectivity fraction to a known splice site for splice sites confirmed by aligned reads.



**bin\_size** An integer scalar specifying the bin size for transcript start and end position binning.

### Value

A named list containing following elements:

**tx\_df** a data frame storing extracted transcript data

**tx\_granges** a GRanges object storing genomic positions of extracted transcript

**tx\_exon\_granges\_list** a GRangesList object storing exon genomic positions of extracted transcript

**tx\_intron\_granges\_list** a GRangesList object storing intron genomic positions of extracted transcript

---

pseudobulk_tcc	<i>Prepare a pseudobulk TCC SummarizedExperiment object</i>
----------------	---

---

### Description

Prepares a pseudobulk TCC SummarizedExperiment from TCC data and given cell labels.

### Usage

```
pseudobulk_tcc(se_tcc, cell_labels)
```

### Arguments

**se\_tcc** A TCC SummarizedExperiment object returned by the [bam\\_to\\_tcc](#) function.

**cell\_labels** A vector or a factor containing cell labels acting as a grouping variable.

### Value

A pseudobulk SummarizedExperiment object containing TCC annotation and quantification data.

---

pseudotime_tcc	<i>Merge TCC values using moving window over pseudotime</i>
----------------	---

---

### Description

Prepares a pseudotime window TCC SummarizedExperiment from TCC data and pseudotime values.

**Usage**

```
pseudotime_tcc(
  se_tcc,
  pseudotime,
  trim = 0,
  window_size = 30,
  window_step = 15
)
```

**Arguments**

se_tcc	A TCC SummarizedExperiment object returned by the <a href="#">bam_to_tcc</a> function.
pseudotime	A numeric vector containing the pseudotime values for each cell. Cells not belonging to the analyzed trajectory should be denoted using NA values.
trim	A numeric scalar specifying the fraction (0 to 0.5) of cells to be trimmed from each end of the pseudotime spectrum.
window_size	An integer scalar specifying the window size.
window_step	An integer scalar specifying the window step.

**Value**

A SummarizedExperiment object containing TCC data for pseudotime windows.

---

tcc_to_gene	<i>Prepare a gene-level SummarizedExperiment object</i>
-------------	---

---

**Description**

Prepares a gene-level SummarizedExperiment from TCC data.

**Usage**

```
tcc_to_gene(se_tcc)
```

**Arguments**

se_tcc	A TCC SummarizedExperiment object returned by a function from the <a href="#">Isosceles-package</a> .
--------	---

**Value**

A SummarizedExperiment object containing gene annotation and quantification data.

---

tcc_to_transcript	<i>Prepare a transcript-level SummarizedExperiment object</i>
-------------------	---

---

### Description

Prepares a transcript-level SummarizedExperiment from TCC data using the EM algorithm.

### Usage

```
tcc_to_transcript(
  se_tcc,
  em.maxiter = 250,
  em.conv = 0.01,
  use_length_normalization = TRUE,
  ncpu = 1
)
```

### Arguments

se_tcc	A TCC SummarizedExperiment object returned by a function from the <a href="#">Isosceles</a> -package.
em.maxiter	An integer scalar specifying the maximum number of EM iterations.
em.conv	A numeric scalar specifying the EM convergence threshold.
use_length_normalization	A logical scalar specifying if normalization using effective transcript lengths should be used during EM.
ncpu	An integer scalar specifying the number of cores to use for multicore parallelization.

### Value

A SummarizedExperiment object containing transcript annotation and quantification data.

---

transcript_to_psi	<i>Prepare a PSI SummarizedExperiment object</i>
-------------------	--

---

### Description

Prepares a PSI (Percent Spliced In) SummarizedExperiment object for the given transcript-level SummarizedExperiment object. PSI values are calculated for the following types of regions:

- **TSS** - transcription start sites
- **TES** - transcription end sites
- **CE** - core exonic regions
- **RI** - retained intronic regions

- **A5** - 5' alternative exonic regions
- **A3** - 3' alternative exonic regions

TSS and TES positions are calculated based on transcripts' binned start and end coordinates extracted from their identifiers.

**Usage**

```
transcript_to_psi(se, ncpu = 1)
```

**Arguments**

se	A transcript-level SummarizedExperiment object returned by the <a href="#">tcc_to_transcript</a> function.
ncpu	An integer scalar specifying the number of cores to use for multicore parallelization.

**Value**

A SummarizedExperiment object containing PSI annotation and quantification data.

# Index

bam\_to\_read\_structures, [3](#), [8](#)

bam\_to\_tcc, [4](#), [5](#), [9](#), [10](#)

export\_gtf, [5](#)

Isosceles-package, [3](#)

neighborhood\_tcc, [5](#)

pheatmap, [6](#)

plot\_psi\_heatmap, [6](#)

plot\_psi\_regions, [7](#)

prepare\_transcripts, [4](#), [8](#)

pseudobulk\_tcc, [9](#)

pseudotime\_tcc, [9](#)

tcc\_to\_gene, [10](#)

tcc\_to\_transcript, [5](#), [7](#), [11](#), [12](#)

transcript\_to\_psi, [6](#), [7](#), [11](#)