

# Package ‘icetea’

January 23, 2026

**Type** Package

**Title** Integrating Cap Enrichment with Transcript Expression Analysis

**Version** 1.28.0

**Description** icetea (Integrating Cap Enrichment with Transcript Expression Analysis) provides functions for end-to-end analysis of multiple 5'-profiling methods such as CAGE, RAMPAGE and MAPCap, beginning from raw reads to detection of transcription start sites using replicates. It also allows performing differential TSS detection between group of samples, therefore, integrating the mRNA cap enrichment information with transcript expression analysis.

**Depends** R (>= 4.0)

**Imports** stats, utils, methods, graphics, grDevices, ggplot2, GenomicFeatures, ShortRead, BiocParallel, Biostrings, S4Vectors, Rsamtools, BiocGenerics, IRanges, GenomicAlignments, GenomicRanges, rtracklayer, SummarizedExperiment, VariantAnnotation, limma, edgeR, csaw, DESeq2, TxDb.Dmelanogaster.UCSC.dm6.ensGene

**Suggests** GenomeInfoDb, knitr, rmarkdown, Rsubread (>= 1.29.0), testthat

**VignetteBuilder** knitr

**biocViews** ImmunoOncology, Transcription, GeneExpression, Sequencing, RNASeq, Transcriptomics, DifferentialExpression

**URL** <https://github.com/vivekbhr/icetea>

**BugReports** <https://github.com/vivekbhr/icetea/issues>

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---

activeChrs

*Match BAM headers bw files and get active chromosome list (from restrict) (written by Aaron Lun, 12 Dec 2014, copied and modified here)*

---

## Description

Match BAM headers bw files and get active chromosome list (from restrict) (written by Aaron Lun, 12 Dec 2014, copied and modified here)

## Usage

```
activeChrs(bam.files, restrict)
```

## Arguments

|           |                                  |
|-----------|----------------------------------|
| bam.files | Character . bam files to check   |
| restrict  | character. Chromosomes to select |

## Value

Vector of selected chromosomes

---

|             |                                                        |
|-------------|--------------------------------------------------------|
| annotateTSS | <i>Annotate the provided Transcription Start Sites</i> |
|-------------|--------------------------------------------------------|

---

## Description

This function annotates the provided TSS bed file to provide the number of TSS falling within the genomic features from a given TxDB object. In order to break ties between overlapping features, the function ranks the features by preference. By default, the following order is used: fiveUTR > promoter > intron > coding > spliceSite > threeUTR > intergenic. A custom order of feature ranks can also be provided.

## Usage

```
annotateTSS(
  tssBED,
  txdb,
  featureRank = c("fiveUTR", "promoter", "intron", "coding", "spliceSite", "threeUTR",
    "intergenic"),
  plotValue = "number",
  outFile = NULL
)
```

## Arguments

|             |                                                                                                                                           |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| tssBED      | A bed file with detected TSS/differential TSS coordinates                                                                                 |
| txdb        | A txdb object.                                                                                                                            |
| featureRank | A vector with features to use for breaking ties, in decending order of preference (highest to lowest),                                    |
| plotValue   | What values to plot (choose from "number", "percent" or NULL for no plot)                                                                 |
| outFile     | Output file name. (filename extension would be used to determine type). If outfile not specified, the plot would be retured on the screen |

## Value

A data.frame with number of TSS falling into each feature

**Examples**

```
# load a txdb object
library("TxDb.Dmelanogaster.UCSC.dm6.ensGene")
library(GenomeInfoDb) # for seqlevelsStyle()
seqlevelsStyle(TxDb.Dmelanogaster.UCSC.dm6.ensGene) <- "ENSEMBL"
# limiting the annotation to X chromosome
seqlevels(TxDb.Dmelanogaster.UCSC.dm6.ensGene) <- "X"

# annotate a given TSS bed file
dir <- system.file("extdata", package = "icetea")
tss <- file.path(dir, "testTSS_merged.bed")
annotations <- annotateTSS(tssBED = tss, TxDb.Dmelanogaster.UCSC.dm6.ensGene,
                          plotValue = "number", outFile = "TSS_annot.pdf")
```

---

|              |                              |
|--------------|------------------------------|
| check_capSet | <i>Check capset validity</i> |
|--------------|------------------------------|

---

**Description**

Check capset validity

**Usage**

```
check_capSet(object)
```

**Arguments**

|        |               |
|--------|---------------|
| object | capset object |
|--------|---------------|

**Value**

boolean

---

|                  |                                                              |
|------------------|--------------------------------------------------------------|
| demultiplexFASTQ | <i>Demultiplex and tag fastq files using sample barcodes</i> |
|------------------|--------------------------------------------------------------|

---

**Description**

Demultiplex and tag fastq files using sample barcodes

**Usage**

```
demultiplexFASTQ(CSobject, outdir, max_mismatch = 0, ncores = 1)

## S4 method for signature 'CapSet'
demultiplexFASTQ(CSobject, outdir, max_mismatch = 0, ncores = 1)
```

**Arguments**

|              |                                                                |
|--------------|----------------------------------------------------------------|
| CSubject     | CapSet object created using <a href="#">newCapSet</a> function |
| outdir       | character. path to output directory                            |
| max_mismatch | integer. maximum allowed mismatches in the sample barcode      |
| ncores       | integer. No. of cores/threads to use                           |

**Value**

de-multiplexed fastq files corresponding to each barcode. The files are written on disk with the corresponding sample names as specified in the CapSet object

**Examples**

```
# load a previously saved CapSet object
cs <- exampleCSubject()

# demultiplex allowing one mismatch in sample indexes
dir.create("demult_fastq")
cs <- demultiplexFASTQ(cs, outdir = "demult_fastq", max_mismatch = 1)
```

---

|               |                                                                                                |
|---------------|------------------------------------------------------------------------------------------------|
| detectDiffTSS | <i>Detect differentially expressed Transcription Start Sites between two conditions (test)</i> |
|---------------|------------------------------------------------------------------------------------------------|

---

**Description**

Detect differentially expressed Transcription Start Sites between two conditions (test)

**Usage**

```
detectDiffTSS(fit, testGroup, contGroup, TSSfile = NULL, MAplot_fdr = NA)

## S4 method for signature 'DGEGLM'
detectDiffTSS(fit, testGroup, contGroup, TSSfile = NULL, MAplot_fdr = NA)

## S4 method for signature 'DESeqDataSet'
detectDiffTSS(fit, testGroup, contGroup, MAplot_fdr = NA)
```

**Arguments**

|            |                                                                                            |
|------------|--------------------------------------------------------------------------------------------|
| fit        | DGEGLM object (output of <a href="#">fitDiffTSS</a> command )                              |
| testGroup  | Test group name                                                                            |
| contGroup  | Control group name                                                                         |
| TSSfile    | The TSS .bed file used for <a href="#">fitDiffTSS</a> command (if method "edgeR" was used) |
| MAplot_fdr | FDR threshold to mark differentially expressed TSS in MAplot (NA = Don't make an MAplot)   |

**Value**

A [GRanges](#) object containing p-values of differential expression for each TSS.

**Examples**

```
# before running this
# 1. Create a CapSet object
# 2. de-multiplex the fastqs
# 3. map them
# 4. filter duplicate reads from mapped BAM
# 5. detect TSS
# 6. fit the diff TSS model.

## Not run:
# load a previously saved DGEGLM object from step 5
csfit <- load("diffTSS_fit.Rdata")
dir <- system.file("extdata", package = "icetea")
# detect differentially expressed TSS between groups (return MA plot)
detectDiffTSS(csfit, testGroup = "mut", controlGroup = "wt",
              tssFile = file.path(dir, "testTSS_merged.bed"), MAplot_fdr = 0.05)

## End(Not run)

## Not run:
# load a previously saved DGEGLM object from step 5
csfit <- load("diffTSS_fit.Rdata")
dir <- system.file("extdata", package = "icetea")
# detect differentially expressed TSS between groups (return MA plot)
detectDiffTSS(csfit, testGroup = "mut", controlGroup = "wt", MAplot_fdr = 0.05)

## End(Not run)
```

---

detectTSS

*Detection of Transcription start sites based on local enrichment*

---

**Description**

Detection of Transcription start sites based on local enrichment

**Usage**

```
detectTSS(
  CSubject,
  groups,
  outfile_prefix = NULL,
  windowSize = 10L,
  sliding = TRUE,
  foldChange = 2,
  mergeLength = 1L,
  restrictChr = NULL,
```

```

    ncores = 1,
    readPos = "start"
)

## S4 method for signature 'CapSet'
detectTSS(
  CSubject,
  groups,
  outfile_prefix = NULL,
  windowSize = 10L,
  sliding = TRUE,
  foldChange = 2,
  mergeLength = 1L,
  restrictChr = NULL,
  ncores = 1,
  readPos = "start"
)

```

### Arguments

|                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CSubject       | CapSet object created using <a href="#">newCapSet</a> function                                                                                                                                                                                                                                                                                                                                                                                                                             |
| groups         | a character vector that contains group name of the sample, for replicate-based TSS calling (see example)                                                                                                                                                                                                                                                                                                                                                                                   |
| outfile_prefix | Output name prefix for the .Rdata file containing window counts, background counts and filtering statistics calculated during TSS detection.                                                                                                                                                                                                                                                                                                                                               |
| windowSize     | Size of the window to bin the genome for TSS detection. By default, a window size of 10 is used for binning the genome, however smaller window sizes can optionally be provided for higher resolution TSS detection. Note that the background size is set to 200x the window size (2kb for 10bp windows) to calculate local enrichment. Subsequently enriched windows are merged, unless the mergeLength is increased.                                                                     |
| sliding        | TRUE/FALSE. Indicating whether or not to use sliding windows. The windows are shifted by length which is half of the specified window length.                                                                                                                                                                                                                                                                                                                                              |
| foldChange     | Numeric. A fold change cutoff of local enrichment to detect the TSS. If the samples have good signal enrichment over background (inspect in genome browser), a low cutoff of 2-fold can be used. For samples with low sequencing depth it's also desirable to have a low cutoff of 2-fold. The final "score" of detected TSS is the mean fold-change of all merged windows that passed the foldChange cutoff. TSSs can therefore also be filtered using this score after detectTSS is run. |
| mergeLength    | Integer. Merge the windows within this distance that pass the foldChange cutoff. Default (1L) means that only subsequently enriched windows would be merged.                                                                                                                                                                                                                                                                                                                               |
| restrictChr    | Chromosomes to restrict the analysis to.                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| ncores         | No. of cores/threads to use                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| readPos        | character. position of read to use. Options are "start", "end" and "center". For TSS detection, the "start" of reads are used (default). But center or end might be useful for detecting RNA-binding proteins (in iCLIP-like data)                                                                                                                                                                                                                                                         |

### Value

.bed files containing TSS position for each group, along with a bed file for consensus (union) TSS sites of all samples.

**Examples**

```
# before running this
# 1. Create a CapSet object
# 2. de-multiplex the fastqs
# 3. map them
# 4. filter duplicate reads from mapped BAM

# load a previously saved CapSet object
cs <- exampleCSobject()
# detect TSS (samples in same group are treated as replicates)
cs <- detectTSS(cs, groups = rep(c("wt", "mut"), each = 2), outfile_prefix = "testTSS",
               foldChange = 6, restrictChr = "X", ncores = 1)
```

---

|             |                                      |
|-------------|--------------------------------------|
| diffQCplots | <i>Make DESeq2 or edgeR QC plots</i> |
|-------------|--------------------------------------|

---

**Description**

Make DESeq2 or edgeR QC plots

**Usage**

```
diffQCplots(method, fit, y, designMat)
```

**Arguments**

|           |                                             |
|-----------|---------------------------------------------|
| method    | one of "DESeq2" or "edgeR"                  |
| fit       | output of fitDiffTSS (if method = "edgeR")  |
| y         | output of fitDiffTSS (if method = "DESeq2") |
| designMat | design matrix (data frame)                  |

**Value**

Sparsity, dispersion and PCA plot (if method = DESeq2), BCV, dispersion and MDS plot (if method = "edgeR")

---

|                 |                                     |
|-----------------|-------------------------------------|
| exampleCSobject | <i>Create example CapSet object</i> |
|-----------------|-------------------------------------|

---

**Description**

Create example CapSet object

**Usage**

```
exampleCSobject(expMethod = "MAPCap")
```

**Arguments**

expMethod      Which experiment method to use (options : "RAMPAGE", "MAPCap")

**Value**

An object of class CapSet

**Examples**

```
cs <- exampleCSobject(expMethod = "MAPCap")
```

---

|           |                                                                 |
|-----------|-----------------------------------------------------------------|
| exportTSS | <i>Export the detected TSS from CapSet object as .bed files</i> |
|-----------|-----------------------------------------------------------------|

---

**Description**

Export the detected TSS from CapSet object as .bed files

**Usage**

```
exportTSS(CSobject, outfile_prefix, pergroup = FALSE, merged = TRUE)
```

```
## S4 method for signature 'CapSet'
```

```
exportTSS(CSobject, outfile_prefix, pergroup = FALSE, merged = TRUE)
```

**Arguments**

CSobject      The modified CapSet object after running [detectTSS](#) function

outfile\_prefix      Prefix (with path) for output .bed files

pergroup      If TRUE, write output per group of samples

merged      If TRUE, write merged bed file (union of all groups)

**Value**

.bed file(s) containing detected TSS.

**Examples**

```
# load a previously saved CapSet object
cs <- exampleCSobject()
# export tss
exportTSS(cs, merged = TRUE, outfile_prefix = "testTSS")
```

---

|                  |                                                                    |
|------------------|--------------------------------------------------------------------|
| filterDuplicates | <i>Filter PCR-duplicates from mapped files using internal UMIs</i> |
|------------------|--------------------------------------------------------------------|

---

## Description

This script considers the read mapping start position and the UMI to determine whether a read is a PCR duplicate. All PCR duplicates are then removed and one entry per read is kept. In case of paired-end reads (MAPCap/RAMPAGE), only one end (R1) is kept after filtering, unless ‘keep-Pairs’ is set to TRUE

## Usage

```
filterDuplicates(CSobject, outdir, ncores = 1, keepPairs = FALSE)

## S4 method for signature 'CapSet'
filterDuplicates(CSobject, outdir, ncores = 1, keepPairs = FALSE)
```

## Arguments

|           |                                                                                                                                                                                               |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CSobject  | an object of class <a href="#">CapSet</a>                                                                                                                                                     |
| outdir    | character. output directory for filtered BAM files                                                                                                                                            |
| ncores    | integer. No. of cores to use                                                                                                                                                                  |
| keepPairs | logical. indicating whether to keep pairs in the paired-end data. (note: the pairs are treated as independent reads during duplicate removal). Also use keepPairs = TRUE for single-end data. |

## Value

modified CapSet object with filtering information. Filtered BAM files are saved in ‘outdir’.

## Examples

```
# before running this
# 1. Create a CapSet object
# 2. de-multiplex the fastqs
# 3. map them

# load a previously saved CapSet object
cs <- exampleCSobject()
# filter duplicate reads from mapped BAM files
dir.create("filtered_bam")
cs <- filterDuplicates(cs, outdir = "filtered_bam")
```

---

|            |                                                                |
|------------|----------------------------------------------------------------|
| filterDups | <i>Filter PCR-duplicates from BAM file using internal UMIs</i> |
|------------|----------------------------------------------------------------|

---

**Description**

Filter PCR-duplicates from BAM file using internal UMIs

**Usage**

```
filterDups(bamFile, outFile, keepPairs)
```

**Arguments**

|           |                                       |
|-----------|---------------------------------------|
| bamFile   | character. Input BAM file             |
| outFile   | character. Output (filtered) BAM file |
| keepPairs | logical. Keep R2 read?                |

**Value**

Filtered BAM file, after PCR duplicate removal

---

|            |                                                                                                     |
|------------|-----------------------------------------------------------------------------------------------------|
| fitDiffTSS | <i>Detect differentially expressed Transcription Start Sites between two conditions (fit model)</i> |
|------------|-----------------------------------------------------------------------------------------------------|

---

**Description**

Detect differentially expressed Transcription Start Sites between two conditions (fit model)

**Usage**

```
fitDiffTSS(
  CSubject,
  TSSfile = NULL,
  groups,
  method = "DESeq2",
  normalization = NULL,
  normFactors = NULL,
  outplots = NULL,
  plotRefSample = NA,
  ncores = 1
)

## S4 method for signature 'CapSet'
fitDiffTSS(
  CSubject,
  TSSfile = NULL,
  groups,
  method = "DESeq2",
```

```

normalization = NULL,
normFactors = NULL,
outplots = NULL,
plotRefSample = NA,
ncores = 1
)

```

### Arguments

|               |                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CSubject      | An object of class <a href="#">CapSet</a>                                                                                                                                                                                                                                                                                                                                                                                                                     |
| TSSfile       | A .bed file with TSS positions to test for differential TSS analysis. If left empty, the union of detected TSS present within the provided CSubject would be plotted.                                                                                                                                                                                                                                                                                         |
| groups        | Character vector indicating the group into which each sample within the CSubject falls. the groups would be use to create a design matrix. As an example, replicates for one condition could be in the same group.                                                                                                                                                                                                                                            |
| method        | Which method to use for differential expression analysis? options are "DESeq2" or "edgeR". If "DESeq2" is chosen, the library size is either estimated via DESeq2 (using "median of ratios") or can be provided via the "normFactors" option below. Setting the "normalization" (below) has no effect in that case.                                                                                                                                           |
| normalization | A character indicating the type of normalization to perform. Options are "windowTMM", "TMM", "RLE", "upperquartile" or NULL (don't compute normalization factors). If "windowTMM" is chosen, the normalization factors are calculated using the TMM method on 10 kb windows of the genome. "TMM" computes TMM normalization using counts from all the evaluated TSSs. If NULL, the external normalization factors can be used (provided using 'normFactors'). |
| normFactors   | external normalization factors (from Spike-Ins, for example).                                                                                                                                                                                                                                                                                                                                                                                                 |
| outplots      | Output pdf filename for plots. If provided, the plots for BCV, dispersion and MDS plot is created and saved in this file.                                                                                                                                                                                                                                                                                                                                     |
| plotRefSample | Name of reference sample to plot for detection of composition bias in the data. Samples could be normalized using one of the provided normalization methods to control for composition bias.                                                                                                                                                                                                                                                                  |
| ncores        | No. of cores/threads to use                                                                                                                                                                                                                                                                                                                                                                                                                                   |

### Value

An object of class [DGEGLM-class](#) or [DESeqDataSet](#)

### Examples

```

# before running this
# 1. Create a CapSet object
# 2. de-multiplex the fastqs
# 3. map them
# 4. filter duplicate reads from mapped BAM
# 5. detect TSS
# 6. fit the diffTSS model
## Not run:
# load a previously saved CapSet object
cs <- exampleCSubject()

# count reads on all TSS (union) and fit a model using replicates within groups

```

```
csfit <- fitDiffTSS(cs, groups = rep(c("wt", "mut"), each = 2), normalization = "internal",
                  outplots = NULL, plotRefSample = "embryo1")
save(csfit, file = "diffTSS_fit.Rdata")

## End(Not run)
```

---

|             |                                   |
|-------------|-----------------------------------|
| getBamFlags | <i>Get flags to read from bam</i> |
|-------------|-----------------------------------|

---

**Description**

Get flags to read from bam

**Usage**

```
getBamFlags(countAll)
```

**Arguments**

|          |                           |
|----------|---------------------------|
| countAll | logical. count all reads? |
|----------|---------------------------|

**Value**

bamFlags

---

|              |                                           |
|--------------|-------------------------------------------|
| getChromBins | <i>Get chromosome bins from BAM files</i> |
|--------------|-------------------------------------------|

---

**Description**

Get chromosome bins from BAM files

**Usage**

```
getChromBins(bamFiles, restrictChr = NULL, binSize)
```

**Arguments**

|             |                                  |
|-------------|----------------------------------|
| bamFiles    | Character. bam files             |
| restrictChr | character. Chromosomes to select |
| binSize     | numeric. Size of bins            |

**Value**

GRanges (bins) for both strands

---

|                 |                                                      |
|-----------------|------------------------------------------------------|
| getChromWindows | <i>Get chromosome sliding windows from BAM files</i> |
|-----------------|------------------------------------------------------|

---

**Description**

Get chromosome sliding windows from BAM files

**Usage**

```
getChromWindows(bamFiles, restrictChr = NULL, binSize, stepSize)
```

**Arguments**

|             |                              |
|-------------|------------------------------|
| bamFiles    | Character vector (bam files) |
| restrictChr | Chromosomes to select        |
| binSize     | Size of bins                 |
| stepSize    | Size of window slide         |

**Value**

GRanges (sliding windows) for both strands

---

|               |                                            |
|---------------|--------------------------------------------|
| getGeneCounts | <i>Get gene-level counts from TSS data</i> |
|---------------|--------------------------------------------|

---

**Description**

Get gene-level counts from TSS data

**Usage**

```
getGeneCounts(
  CSubject,
  transcriptGRL,
  regionAroundTSS = 500,
  outfile = NA,
  ncores = 1
)

## S4 method for signature 'CapSet'
getGeneCounts(
  CSubject,
  transcriptGRL,
  regionAroundTSS = 500,
  outfile = NA,
  ncores = 1
)
```

**Arguments**

|                 |                                                                                |
|-----------------|--------------------------------------------------------------------------------|
| CSubject        | The <a href="#">CapSet</a> object to use.                                      |
| transcriptGRL   | A GRangesList object containing transcripts, created using transcriptsBy(txdb) |
| regionAroundTSS | integer, indicating how many bases downstream of TSS to count                  |
| outfile         | character. Tab-separated output file name (if required)                        |
| ncores          | integer. No. of cores/threads to use                                           |

**Value**

data.frame with gene-level counts for all genes in the txdb object

**Examples**

```
# load a txdb object
library("TxDb.Dmelanogaster.UCSC.dm6.ensGene")
library(GenomeInfoDb) # for seqlevelsStyle()
seqlevelsStyle(TxDb.Dmelanogaster.UCSC.dm6.ensGene) <- "ENSEMBL"

# get transcripts by gene (only X chromosome, for simplicity)
seqlevels(TxDb.Dmelanogaster.UCSC.dm6.ensGene) <- "X"
dm6trans <- transcriptsBy(TxDb.Dmelanogaster.UCSC.dm6.ensGene, "gene")

# load a CapSet object
cs <- exampleCSubject()
# get gene counts, counting reads around 500 bp of the TSS
gcounts <- getGeneCounts(cs, dm6trans)
```

---

getMCparams

*Get platform-specific multicore params*


---

**Description**

Get platform-specific multicore params

**Usage**

```
getMCparams(cores)
```

**Arguments**

|       |                               |
|-------|-------------------------------|
| cores | integer. No. of cores to use. |
|-------|-------------------------------|

**Value**

BPPARAM object

---

|                |                                                           |
|----------------|-----------------------------------------------------------|
| getNormFactors | <i>Calculate normalization factors from CapSet object</i> |
|----------------|-----------------------------------------------------------|

---

### Description

Calculate normalization factors from CapSet object

### Usage

```
getNormFactors(CSobject, features, method = "TMM", ...)
```

```
## S4 method for signature 'CapSet'
```

```
getNormFactors(CSobject, features, method = "TMM", ...)
```

### Arguments

|          |                                                                                  |
|----------|----------------------------------------------------------------------------------|
| CSobject | An object of class <a href="#">CapSet</a>                                        |
| features | A <a href="#">GRanges-class</a> .object to count the reads on.                   |
| method   | Method to use for normalization. Options : "TMM", "RLE", "upperquartile", "none" |
| ...      | Additional arguments passed to <a href="#">calcNormFactors</a>                   |

### Value

Numeric vector of calculated normalization factors.

### Examples

```
# load a txdb object
library("TxDb.Dmelanogaster.UCSC.dm6.ensGene")
library(GenomeInfoDb) # for seqlevelsStyle()
seqlevelsStyle(TxDb.Dmelanogaster.UCSC.dm6.ensGene) <- "ENSEMBL"

# get genes (only X chromosome, for simplicity)
seqlevels(TxDb.Dmelanogaster.UCSC.dm6.ensGene) <- "X"
dm6genes <- genes(TxDb.Dmelanogaster.UCSC.dm6.ensGene)

# get norm factors by counting reads on genes
cs <- exampleCSobject()
normfacs <- getNormFactors(cs, dm6genes, method = "RLE")
```

---

|          |                                                           |
|----------|-----------------------------------------------------------|
| getranks | <i>Assign feature ranks on a VariantAnnotation output</i> |
|----------|-----------------------------------------------------------|

---

### Description

Assign feature ranks on a VariantAnnotation output

### Usage

```
getranks(x, rank_vec)
```

**Arguments**

|          |                               |
|----------|-------------------------------|
| x        | output from VariantAnnotation |
| rank_vec | the pre-set vector of ranks   |

**Value**

A vector of ranks of length = length of input features

---

|              |                                                                        |
|--------------|------------------------------------------------------------------------|
| get_newfastq | <i>Get data to create new ShortReadQ object after barcode trimming</i> |
|--------------|------------------------------------------------------------------------|

---

**Description**

Get data to create new ShortReadQ object after barcode trimming

**Usage**

```
get_newfastq(type, fq_R1, fq_R2)
```

**Arguments**

|       |                                         |
|-------|-----------------------------------------|
| type  | character. expType of the CapSet object |
| fq_R1 | character. fastq Read 1                 |
| fq_R2 | character. fastq Read 2                 |

**Value**

A list with new R1 and R2 sequence, quality, barcode string and sample id

---

|         |                                                  |
|---------|--------------------------------------------------|
| mapCaps | <i>Map the data from 5' profiling techniques</i> |
|---------|--------------------------------------------------|

---

**Description**

Map the data from 5' profiling techniques

**Usage**

```
mapCaps(
  CSubject,
  genomeIndex,
  outdir,
  externalGTF = NULL,
  ncores = 1,
  logfile = NULL
)

## S4 method for signature 'CapSet'
mapCaps(
```

```

CSubject,
genomeIndex,
outdir,
externalGTF = NULL,
ncores = 1,
logfile = NULL
)

```

### Arguments

|             |                                                                                                         |
|-------------|---------------------------------------------------------------------------------------------------------|
| CSubject    | An object of class <a href="#">CapSet</a>                                                               |
| genomeIndex | character. Path to the Subread index file. Should end with the basename of the index.                   |
| outdir      | character. Output directory path                                                                        |
| externalGTF | character. provide external annotation file in ‘GTF’ format , if present to increase alignment accuracy |
| ncores      | integer. Number of cores/threads to use for mapping.                                                    |
| logfile     | character. A log file to write the processing message.                                                  |

### Value

modified CapSet object with mapping information. Mapped and sorted BAM files are saved in ‘outdir’.

### Examples

```

## Not run:
# before mapping :
# 1. Create a CapSet object
# 2. de-multiplex the fastqs

# load a previously saved CapSet object
cs <- exampleCSubject()

# map the data (not available on windows)
library(Rsubread)
dir.create("bam")
buildindex(basename = "dm6", reference = "/path/to/dm6_genome.fa")
cs <- mapCaps(cs, genomeIndex = "dm6", outdir = "bam", nthreads = 10)

## End(Not run)

```

## Description

The function creates an object of class 'CapSet', used for the TSS analysis. A CapSet object can be created using the the raw, multiplexed fastq files along with the list of sample indexes and corresponding sample names. In case the files are already de-multiplexed or mapped, the CapSet object can also be created using the path to demultiplexed fastq/mapped or filtered BAM files, along with corresponding sample names. In these cases statistics and operations for the missing files would not be possible.

## Usage

```
newCapSet(
  expMethod,
  fastq_R1 = NULL,
  fastq_R2 = NULL,
  idxList = NULL,
  sampleNames,
  demult_R1 = NA,
  demult_R2 = NA,
  mapped_file = NA,
  filtered_file = NA,
  paired_end = TRUE
)
```

## Arguments

|               |                                                                         |
|---------------|-------------------------------------------------------------------------|
| expMethod     | experiment method ('CAGE', 'RAMPAGE' or 'MAPCap')                       |
| fastq_R1      | path for Read R1 (or file path for single end reads)                    |
| fastq_R2      | path for Read R2 (for paired end reads)                                 |
| idxList       | a vector of index sequences (for demultiplexing)                        |
| sampleNames   | (required) a vector of sample names corresponding to the provided files |
| demult_R1     | a vector of file paths for demultiplexed R1 reads                       |
| demult_R2     | a vector of file paths for demultiplexed R2 reads                       |
| mapped_file   | a vector of file paths for mapped BAM files.                            |
| filtered_file | a vector of file paths for de-duplicated BAM files.                     |
| paired_end    | logical, indicating whether the data is paired end                      |

## Value

An object of class CapSet

## Slots

|              |                                                                                      |
|--------------|--------------------------------------------------------------------------------------|
| fastqType    | Type of fastq ('single' or 'paired')                                                 |
| fastq_R1     | Path to R1 fastq                                                                     |
| fastq_R2     | Path to R1 fastq (for paired-end data)                                               |
| expMethod    | Name of protocol (RAMPGE or MAPCap)                                                  |
| sampleInfo   | A DataFrame object created using information from <a href="#">newCapSet</a> function |
| tss_detected | A GRangesList object of detected TSS                                                 |

**Examples**

```
# list of barcode IDs
idxlist <- c("CAAGTG", "TTAGCC", "GTGGAA", "TGTGAG")

dir <- system.file("extdata", package="icetea")
# corresponding sample names
fnames <- c("embryo1", "embryo2", "embryo3", "embryo4")

## CapSet object from raw (multiplexed) fastq files
cs <- newCapSet(expMethod = 'MAPCap',
  fastq_R1 = file.path(dir, 'mapcap_test_R1.fastq.gz'),
  fastq_R2 = file.path(dir, 'mapcap_test_R2.fastq.gz'),
  idxList = idxlist,
  sampleNames = fnames)

## CapSet object from mapped BAM files
bams <- list.files(file.path(dir, 'bam'), pattern = '.bam$', full.names = TRUE)
cs <- newCapSet(expMethod = 'MAPCap',
  mapped_file = bams,
  sampleNames = fnames)
```

---

numReadsInBed

*Count the number of reads in a given GRanges*


---

**Description**

Count the number of reads in a given GRanges

**Usage**

```
numReadsInBed(regions, bams = NA, countall = FALSE)
```

**Arguments**

|          |                                                                      |
|----------|----------------------------------------------------------------------|
| regions  | The GRanges object to count reads in.                                |
| bams     | character. path to bam files from where the reads have to be counted |
| countall | logical. whether to keep both reads of paired-end data               |

**Value**

Total counts within given ranges per BAM file.

---

|               |                                        |
|---------------|----------------------------------------|
| plotPrecision | <i>Plotprecision background script</i> |
|---------------|----------------------------------------|

---

**Description**

Plotprecision background script

**Usage**

```
plotPrecision(ref, tssData, distCut)
```

**Arguments**

|         |                                                          |
|---------|----------------------------------------------------------|
| ref     | GRanges. reference ranges to compare the precision with. |
| tssData | GRangesList object with TSS detected per sample          |
| distCut | integer. max distance cutoff                             |

**Value**

ggplot object

---

|               |                                                    |
|---------------|----------------------------------------------------|
| plotReadStats | <i>Plot read statistics from the CapSet object</i> |
|---------------|----------------------------------------------------|

---

**Description**

Plot read statistics from the CapSet object

**Usage**

```
plotReadStats(  
  CSubject,  
  plotType = "dodge",  
  plotValue = "numbers",  
  outFile = NULL  
)  
  
## S4 method for signature 'CapSet'  
plotReadStats(  
  CSubject,  
  plotType = "dodge",  
  plotValue = "numbers",  
  outFile = NULL  
)
```

**Arguments**

|           |                                                                                                                                                                                           |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CSubject  | The <a href="#">CapSet</a> object                                                                                                                                                         |
| plotType  | character. The type of plot to make. Choose from "stack" or "dodge" for either a stacked barchart, or a bar chart with "dodged" positions (analogous to ggplot)                           |
| plotValue | character. What values to plot. Choose from "numbers" or "proportions". If "proportions" is selected, the proportion of reads w.r.t total demultiplexed reads per sample would be plotted |
| outFile   | character. Output file name. (filename extension would be used to determine type). If outfile not specified, the plot would be returned on the screen                                     |

**Value**

A ggplot object, or a file. Plot showing the number/proportion of reads in each category, per sample

**Examples**

```
# load a previously saved CapSet object
cs <- exampleCSubject()
plotReadStats(cs, plotType = "dodge", plotValue = "numbers", outFile = "test_numbers.pdf")
```

---

|                  |                                                                        |
|------------------|------------------------------------------------------------------------|
| plotTSSprecision | <i>Compare the precision of TSS detection between multiple samples</i> |
|------------------|------------------------------------------------------------------------|

---

**Description**

Plot precision of TSS detection from multiple samples (bed files) with respect to a given reference annotation.

Plot precision of TSS detection from multiple samples present within a [CapSet](#) object, with respect to a given reference annotation.

**Usage**

```
plotTSSprecision(
  reference,
  detectedTSS,
  distanceCutoff = 500,
  outFile = NULL,
  ...
)

## S4 method for signature 'GRanges,character'
plotTSSprecision(
  reference,
  detectedTSS,
  distanceCutoff = 500,
  outFile = NULL,
  sampleNames
)
```

```
## S4 method for signature 'GRanges, CapSet'
plotTSSprecision(
  reference,
  detectedTSS,
  distanceCutoff = 500,
  outFile = NULL,
  ...
)
```

### Arguments

|                |                                                                                                                                                                                   |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| reference      | Reference Transcripts/Genes as a <a href="#">GRanges</a> object                                                                                                                   |
| detectedTSS    | Either a <a href="#">CapSet</a> object with TSS information (after running <a href="#">detectTSS</a> ) or a character vector with paths to the BED files containing detected TSSs |
| distanceCutoff | integer. Maximum distance (in base pairs) from reference TSS to plot                                                                                                              |
| outFile        | character. Output file name (filename extension would be used to determine type) If outfile not specified, the plot would be returned on the screen                               |
| ...            | Additional arguments                                                                                                                                                              |
| sampleNames    | character. Labels for input samples (in the same order as the input bed files)                                                                                                    |

### Value

A ggplot object, or a file. Plot showing percent of TSS detected per sample with respect to their cumulative distance to TSS of the provided reference

### Examples

```
# load a txdb object
suppressMessages(library("TxDb.Dmelanogaster.UCSC.dm6.ensGene"))
library(GenomeInfoDb) # for seqlevelsStyle()
seqlevelsStyle(TxDb.Dmelanogaster.UCSC.dm6.ensGene) <- "ENSEMBL"
transcripts <- transcripts(TxDb.Dmelanogaster.UCSC.dm6.ensGene)

# Plotting the precision using a pre computed set of TSS (.bed files) :

tssfile <- system.file("extdata", "testTSS_merged.bed", package = "icetea")
plotTSSprecision(reference = transcripts, detectedTSS = tssfile,
  sampleNames = "testTSS", distanceCutoff = 500,
  outFile = "TSS_detection_precision.png")

## Plotting the precision using a CapSet object :

library("TxDb.Dmelanogaster.UCSC.dm6.ensGene")
library(GenomeInfoDb) # for seqlevelsStyle()
seqlevelsStyle(TxDb.Dmelanogaster.UCSC.dm6.ensGene) <- "ENSEMBL"
# only use chrX to make the analysis faster
seqlevels(TxDb.Dmelanogaster.UCSC.dm6.ensGene) <- "X"
transcripts <- transcripts(TxDb.Dmelanogaster.UCSC.dm6.ensGene)

# load a previously saved CapSet object
cs <- exampleCSobject()
# plot
plotTSSprecision(reference = transcripts, detectedTSS = cs,
  outFile = "TSS_detection_precision.png")
```

---

|           |                                                   |
|-----------|---------------------------------------------------|
| readsTo3p | <i>preprocess reads to count only 3' overlaps</i> |
|-----------|---------------------------------------------------|

---

**Description**

preprocess reads to count only 3' overlaps

**Usage**

```
readsTo3p(reads, width = 1, fix = "end")
```

**Arguments**

|       |                             |
|-------|-----------------------------|
| reads | GAlignment object to resize |
| width | integer. New read length    |
| fix   | character. 'Start' for 5'   |

**Value**

Resized reads as GRanges

---

|           |                                                   |
|-----------|---------------------------------------------------|
| readsTo5p | <i>preprocess reads to count only 5' overlaps</i> |
|-----------|---------------------------------------------------|

---

**Description**

preprocess reads to count only 5' overlaps

**Usage**

```
readsTo5p(reads, width = 1, fix = "start")
```

**Arguments**

|       |                             |
|-------|-----------------------------|
| reads | GAlignment object to resize |
| width | integer. New read length    |
| fix   | character. 'Start' for 5'   |

**Value**

Resized reads as GRanges

---

|               |                                                       |
|---------------|-------------------------------------------------------|
| readsToCenter | <i>preprocess reads to count only center overlaps</i> |
|---------------|-------------------------------------------------------|

---

**Description**

preprocess reads to count only center overlaps

**Usage**

```
readsToCenter(reads, width = 1, fix = "center")
```

**Arguments**

|       |                             |
|-------|-----------------------------|
| reads | GAlignment object to resize |
| width | integer. New read length    |
| fix   | character. 'Start' for 5'   |

**Value**

Resized reads as GRanges

---

|            |                                                                   |
|------------|-------------------------------------------------------------------|
| sampleInfo | <i>Retrieve and replace sample information of a CapSet object</i> |
|------------|-------------------------------------------------------------------|

---

**Description**

Retrieve and replace sample information of a CapSet object

**Usage**

```
sampleInfo(object, ...)

sampleInfo(object, ...) <- value

## S4 method for signature 'CapSet'
sampleInfo(object)

## S4 replacement method for signature 'CapSet'
sampleInfo(object) <- value
```

**Arguments**

|        |                                   |
|--------|-----------------------------------|
| object | The <a href="#">CapSet</a> object |
| ...    | Additional options                |
| value  | Replacement DataFrame object      |

**Value**

sample information data.frame

**Examples**

```
# load a previously saved CapSet object
cs <- exampleCSobject()
# get sampleinfo
si <- sampleInfo(cs)
# modify
si$samples <- paste0("sample_", seq_along(1:nrow(si)) )
# replace
sampleInfo(cs) <- si
```

splitBAM\_byIndex

*Split the composite BAM file using internal indexes (MAPCap)***Description**

Split the composite BAM file using internal indexes (MAPCap)

**Usage**

```
splitBAM_byIndex(
  bamFile,
  index_list,
  outfile_list,
  max_mismatch = 0,
  ncores = 1
)
```

**Arguments**

|              |                                                                                         |
|--------------|-----------------------------------------------------------------------------------------|
| bamFile      | character. Path to a mapped BAM file                                                    |
| index_list   | character. A list of indexes for splitting                                              |
| outfile_list | character. A list of output file names (with order corresponding to that of index_list) |
| max_mismatch | integer. No. of mismatches allowed in index (maximum 1 recommended)                     |
| ncores       | integer. Number of cores to use for parallel processing                                 |

**Value**

Filtered files

**Examples**

```
bam <- system.file("extdata", "bam/embryo1.bam", package = "icetea")
splitBAM_byIndex(bamFile = bam,
  index_list = c("CAAGTG", "CAAGTT"),
  outfile_list = c("test_filt1.bam", "test_filt2.bam"),
  ncores = 1)
```

---

|                     |                                                                           |
|---------------------|---------------------------------------------------------------------------|
| splitBAM_byRepindex | <i>Split the composite BAM file using replicate indexes (MAPCap data)</i> |
|---------------------|---------------------------------------------------------------------------|

---

**Description**

Split the composite BAM file using replicate indexes (MAPCap data)

**Usage**

```
splitBAM_byRepindex(bamFile, outfile_prefix, ncores = 1)
```

**Arguments**

|                |                                                                           |
|----------------|---------------------------------------------------------------------------|
| bamFile        | character. Path to a mapped BAM file                                      |
| outfile_prefix | character. prefix for output file (replicates IDs will be added as RR/YY) |
| ncores         | integer. Number of cores to use for parallel processing                   |

**Value**

Filtered files by replicate Index

**Examples**

```
bam <- system.file("extdata", "bam/embryo1.bam", package = "icetea")
splitBAM_byRepindex(bamFile = bam, outfile_prefix = "testSplit", ncores = 1)
```

---

|            |                                                     |
|------------|-----------------------------------------------------|
| splitranks | <i>Get features with the best rank for each TSS</i> |
|------------|-----------------------------------------------------|

---

**Description**

Get features with the best rank for each TSS

**Usage**

```
splitranks(x)
```

**Arguments**

|   |                    |
|---|--------------------|
| x | output of getranks |
|---|--------------------|

**Value**

A data frame with counts

---

|             |                                           |
|-------------|-------------------------------------------|
| split_fastq | <i>Split paired-end fastq by barcodes</i> |
|-------------|-------------------------------------------|

---

**Description**

Split paired-end fastq by barcodes

**Usage**

```
split_fastq(
  expType,
  idx_name,
  outfile_R1,
  outfile_R2,
  fastq_R1,
  fastq_R2,
  max_mismatch
)
```

**Arguments**

|              |                                                      |
|--------------|------------------------------------------------------|
| expType      | character. experiment type (RAMPAGE, MAPCap or CAGE) |
| idx_name     | character. barcode ID                                |
| outfile_R1   | character. output fastq file : Read 1                |
| outfile_R2   | character. output fastq file : Read 2                |
| fastq_R1     | character. input fastq file : Read 1                 |
| fastq_R2     | character. input fastq file : Read 2                 |
| max_mismatch | integer. max allowed mismatches                      |

**Value**

kept reads corresponding to each barcode.

---

|                 |                                    |
|-----------------|------------------------------------|
| strandBinCounts | <i>Perform stranded Bin counts</i> |
|-----------------|------------------------------------|

---

**Description**

Perform stranded Bin counts

**Usage**

```
strandBinCounts(
  bam.files,
  restrictChrs,
  bam_param,
  bp_param,
  window_size,
  sliding = FALSE,
  func
)
```

**Arguments**

|              |                                         |
|--------------|-----------------------------------------|
| bam.files    | character vector. BAM files to use      |
| restrictChrs | character vector. chromosomes to use    |
| bam_param    | ScanBAMParams                           |
| bp_param     | BPPARAM                                 |
| window_size  | integer. size of window to use          |
| sliding      | logical. perform sliding window counts? |
| func         | function to preprocess reads            |

**Value**

RangedSE object with forward and reverse strand counts

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