Package: diffUTR (via r-universe)

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Type Package

Title diffUTR: Streamlining differential exon and 3' UTR usage

Version 1.21.0

Depends R (>= 4.0)

Description The diffUTR package provides a uniform interface and plotting functions for limma/edgeR/DEXSeq -powered differential bin/exon usage. It includes in addition an improved version of the limma::diffSplice method. Most importantly, diffUTR further extends the application of these frameworks to differential UTR usage analysis using poly-A site databases.

Imports S4Vectors, SummarizedExperiment, limma, edgeR, DEXSeq, GenomicRanges, Rsubread, ggplot2, rtracklayer, ComplexHeatmap, ggrepel, stringi, methods, stats, GenomeInfoDb, dplyr, matrixStats, IRanges, ensembldb, viridisLite

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Contents

addNormalizedAssays . . . 2
countFeatures . . . 3
deuBinPlot . . . 4
diffSplice2 . . . 5
diffSpliceDGEWrapper . . . 6
example_bin_se . . . 7
example_gene_annotation . . . 8
geneBinHeatmap . . . 8
geneLevelStats . . . 9
plotTopGenes . . . 11
prepareBins . . . 12
rn6_PAS . . . 13
simesAggregation . . . 13

Index **14**

`addNormalizedAssays` *addNormalizedAssays*

Description

addNormalizedAssays

Usage

```
addNormalizedAssays(se, readLength = 50L)
```

Arguments

`se`	A bin-wise 'SummarizedExperiment' as produced by `countFeatures`
`readLength`	Used as a minimum width to estimate read density (default 50).

Value

The 'se' object with populated 'logcpm' and 'logNormDensity' assays.

Examples

```
data(example_bin_se)
example_bin_se <- addNormalizedAssays(example_bin_se)
```

countFeatures *countFeatures*

Description

countFeatures

Usage

```
countFeatures(
  bamfiles,
  bins,
  strandSpecific = 0,
  readLength = 50L,
  allowMultiOverlap = TRUE,
  inclNormalized = TRUE,
  tmpDir = tempdir(),
  ...
)
```

Arguments

bamfiles	A vector of paths to bam files
bins	A GRanges of bins in which to count reads (or path to a rds file containing such an object
strandSpecific	Passed to 'Rsubread::featureCounts'
readLength	Used as a minimum width to estimate read density.
allowMultiOverlap	Passed to 'Rsubread::featureCounts'
inclNormalized	Logical; whether to include normalized assays (needed for plotting)
tmpDir	Passed to 'Rsubread::featureCounts'
...	Passed to 'Rsubread::featureCounts'

Value

A RangedSummarizedExperiment-class

Examples

```
data("example_gene_annotation", package="diffUTR")
bins <- prepareBins(example_gene_annotation)
bam_files <- list.files(system.file("extdata", package="diffUTR"),
                        pattern="bam$", full=TRUE)
# not run
# se <- countFeatures(bam_files, bins, verbose=FALSE)
```

deuBinPlot *deuBinPlot*

Description

deuBinPlot

Usage

```
deuBinPlot(
  se,
  gene,
  type = c("summary", "condition", "sample"),
  intronSize = 2,
  exonSize = c("sqrt", "linear", "log"),
  y = NULL,
  condition = NULL,
  size = "type",
  lineSize = 1,
  colour = NULL,
  alpha = NULL,
  removeAmbiguous = TRUE,
  minDensityRatio = 0.1
)
```

Arguments

se	A bin-wise SummarizedExperiment as produced by countFeatures and including bin-level tests (i.e. having been passed through one of the DEU wrappers such as diffSpliceWrapper or DEXSeqWrapper)
gene	The gene of interest
type	Either 'summary' (plot DEU summary), 'sample' (plot sample-wise data), or 'condition' (plot data aggregate by condition)
intronSize	Intron plot size. If <=3, intron size will be this fraction of the mean exon size. If >3, each intron will have the given size.
exonSize	Scaling for exon sizes, either 'sqrt', 'log', or 'linear'.
y	Value to plot on the y-axis. If 'type="summary"', this should be a column of 'rowData(se)', otherwise should be an assay name of 'se'.
condition	The colData column containing the samples' condition.
size	rowData variable to use to determine the thickness of the bins.
lineSize	Size of the line connecting the bins. Use 'lineSize=0' to omit the line.
colour	rowData variable to use to determine the colour of the bins. If 'type="condition"', can also be "condition"; if 'type="sample"' can be any colData column.
alpha	Alpha level, passed to ggplot.

removeAmbiguous
: Logical; whether to remove bins that are gene-ambiguous (i.e. overlap multiple genes).

minDensityRatio
: Minimum ratio of read density (with respect to the gene's average) for a bin to be plotted.

Value

A ggplot object

Examples

```
data(example_bin_se)
se <- diffSpliceWrapper(example_bin_se, ~condition)
deuBinPlot(se, "Jund")
```

---

diffSplice2 *diffSplice2*

Description

This is a small improvement to the diffSplice function written by Gordon Smyth and Charity Law.

Usage

```
diffSplice2(fit, geneid, exonid = NULL, robust = FALSE, verbose = TRUE)
```

Arguments

fit	an MArrayLM-class fitted model object produced by lmFit or 'contrasts.fit', with rows corresponding to exons.
geneid	gene identifiers (as in diffSplice)
exonid	exon identifiers (as in diffSplice)
robust	logical, should the estimation of the empirical Bayes prior parameters be robustified against outlier sample variances?
verbose	logical, if TRUE will output some diagnostic information

Value

An MArrayLM-class object containing both exon level and gene level tests. Results are sorted by geneid and by exonid within gene.

Examples

```
library(SummarizedExperiment)
library(edgeR)
data(example_bin_se)
se <- example_bin_se
design <- model.matrix(~condition, data=as.data.frame(colData(se)))
dds <- calcNormFactors(DGEList(assays(se)$counts))
dds <- voom(dds, design)
dds <- lmFit(dds, design)
res <- diffSplice2(dds, geneid=rowData(se)$gene, exonid=row.names(se))
topSplice(res)
```

`diffSpliceDGEWrapper` *DEUwrappers*

Description

Wrappers around commonly-used DEU methods (`diffSpliceDGE`, `DEXSeq` and an improved version of `diffSplice`

Usage

```
diffSpliceDGEWrapper(
  se,
  design,
  coef = NULL,
  QLF = TRUE,
  robust = TRUE,
  countFilter = TRUE,
  excludeTypes = NULL
)

diffSpliceWrapper(
  se,
  design,
  coef = NULL,
  robust = TRUE,
  improved = TRUE,
  countFilter = TRUE,
  excludeTypes = NULL
)

DEXSeqWrapper(
  se,
  design = ~sample + exon + condition:exon,
  reducedModel = ~sample + exon,
  excludeTypes = NULL,
```

```
  ...
)
```

Arguments

se	A bin-wise SummarizedExperiment as produced by countFeatures
design	A formula (using columns of 'colData(se)') or (for 'diffSpliceWrapper' or 'diffSpliceDGEWrapper' only) a model.matrix.
coef	The coefficient to be tested (ignored for 'DEXSeqWrapper').
QLF	Logical; whether to use edgeR's quasi-likelihood negative binomial (applicable only to 'diffSpliceDGEWrapper').
robust	Logical; whether to use robust fitting for the dispersion trend (ignored for 'DEXSeqWrapper').
countFilter	Logical; whether to filter out low-count bins (ignored for 'DEXSeqWrapper').
excludeTypes	A vector of bin types to ignore for testing. To test for any kind of differential usage, leave empty. To test for differential UTR usage, use 'excludeTypes=c("CDS","noncoding")' (or see geneLevelStats for more options).
improved	Logical; whether to use diffSplice2 instead of the original diffSplice (default TRUE).
reducedModel	A reduced formula (applicable only to 'DEXSeqWrapper').
...	Further arguments (passed to 'testForDEU' and 'estimateExonFoldChanges') of 'DEXSeq'. Can for instance be used to enable multithreading, by passing 'BPPARAM=BiocParallel::MulticoreParam(ncores)'.

Value

The 'se' object with additional rowData columns contain bin (i.e. exon) -level statistics, and a metadata slot containing gene level p-values.

Examples

```
library(SummarizedExperiment)
data(example_bin_se)
se <- diffSpliceWrapper(example_bin_se, ~condition)
head(rowData(se))
```

---

example_bin_se *Example bin-level 'RangedSummarizedExperiment'*

Description

An object produced by countFeatures containing small subset of genes from mouse hippocampal slices undergoing Forskolin-induced long-term potentiation (GSE84643).

Value

a 'RangedSummarizedExperiment'

References

https://www.nature.com/articles/s41598-017-17407-w

`example_gene_annotation` *Example gene annotation*

Description

An example gene annotation containing only a small subset of mouse genes.

Value

a 'GRanges' object

`geneBinHeatmap` *geneBinHeatmap*

Description

A wrapper around 'ComplexHeatmap'.

Usage

```
geneBinHeatmap(
  se,
  gene,
  what = NULL,
 anno_rows = c("type", "logWidth", "meanLogDensity", "log10PValue", "geneAmbiguous"),
  anno_columns = c(),
  anno_colors = list(),
  removeAmbiguous = FALSE,
  merge_legends = TRUE,
  cluster_columns = FALSE,
  minDensityRatio = 0.1,
  left_annotation = NULL,
  top_annotation = NULL,
  ...
)
```

Arguments

se	A bin-wise SummarizedExperiment as produced by countFeatures
gene	The gene of interest
what	Type of values (i.e. assay) to plot
anno_rows	Row annotation columns (i.e. columns of 'rowData(se)') to plot
anno_columns	Column annotation columns (i.e. columns of 'colData(se)') to plot
anno_colors	Annotation colors, as a list named with the row/column annotations, see 'SingleAnnotation' for details. Ignored if 'left_annotation' and/or 'top_annotation' are given directly.
removeAmbiguous	Logical; whether to remove bins that are gene-ambiguous (i.e. overlap multiple genes).
merge_legends	Logical; whether to merge legends. This effectively calls 'draw(..., merge_legends=TRUE)' around the heatmap.
cluster_columns	Logical; whether to cluster columns (passed to Heatmap)
minDensityRatio	Minimum ratio of read density (with respect to the gene's average) for a bin to be plotted.
left_annotation	Passed to Heatmap, overrides 'anno_rows'.
top_annotation	Passed to Heatmap, overrides 'anno_columns'.
...	Passed to 'ComplexHeatmap' (see Heatmap)

Value

A Heatmap

Examples

```
data(example_bin_se)
se <- diffSpliceWrapper(example_bin_se, ~condition)
geneBinHeatmap(se, "Jund")
```

---

geneLevelStats *geneLevelStats*

Description

Aggregates bin-level statistics to the gene-level

Usage

```
geneLevelStats(
  se,
  coef = NULL,
  excludeTypes = NULL,
  includeTypes = NULL,
  returnSE = TRUE,
  minDensityRatio = 0.1,
  minWidth = 20,
  excludeGeneAmbiguous = TRUE
)
```

Arguments

se	A 'RangedSummarizedExperiment' containing the results of one of the DEU wrappers.
coef	The coefficients tested (if the model included more than one term).
excludeTypes	Vector of bin types to exclude.
includeTypes	Vector of bin types to include (overrides 'excludeTypes')
returnSE	Logical; whether to return the updated 'se' object (default), or the gene-level table.
minDensityRatio	Minimum ratio of read density (with respect to the gene's average) for a bin to be included.
minWidth	Minimum bin width to include
excludeGeneAmbiguous	Logical; whether to exclude bins which are ambiguous (i.e. can be from different genes)

Value

If 'returnSE=TRUE' (default), returns the 'se' object with an updated 'metadata(se)$geneLevel' slot, otherwise returns the gene-level data.frame.

Examples

```
library(SummarizedExperiment)
data(example_bin_se)
se <- diffSpliceWrapper(example_bin_se, ~condition)
se <- geneLevelStats(se, includeTypes="3UTR")
head(metadata(se)$geneLevel)
```

plotTopGenes *plotTopGenes*

Description

plotTopGenes

Usage

```
plotTopGenes(se, n = 10, FDR = 0.05, diffUTR = FALSE, alpha = 1, ...)
```

Arguments

se	A bin-wise SummarizedExperiment as produced by countFeatures and including bin-level tests (i.e. having been passed through one of the DEU wrappers such as diffSpliceWrapper or DEXSeqWrapper)
n	The maximum number of genes for which to plot labels
FDR	The FDR threshold above which to plot labels
diffUTR	Logical; if FALSE, uses absolute coefficients (appropriate for normal differential exon usage); if TRUE, uses non-absolute (ie changes should be in the same direction across significant bins) and width-weighted scores (i.e. larger bins have more weight) – this is relevant only when testing UTR usage.
alpha	Points transparency
...	Passed to geom_label_repel; this can for instance be used to increase 'max.overlaps' when not all desired gene labels are displayed)

Value

A ggplot

Examples

```
data(example_bin_se)
se <- diffSpliceWrapper(example_bin_se, ~condition)
plotTopGenes(se)
```

prepareBins *prepareBins*

Description

prepareBins

Usage

```
prepareBins(
  g,
  APA = NULL,
  onlyMainChr = TRUE,
  removeAntisense = TRUE,
  chrStyle = NULL,
  maxUTRbinSize = 15000,
  codingOnly = FALSE,
  genewise = FALSE,
  stranded = FALSE,
  verbose = TRUE
)
```

Arguments

g	A GRanges (or path to RDS file containing a GRanges) or path to a gtf file or EnsDb object containing the gene annotation.
APA	A GRanges (or path to a GRanges in RDS format) or bed file containing the alternative poly-A site database
onlyMainChr	Logical; whether to keep only main chromosomes
removeAntisense	Logical; whether to remove antisense APA sites
chrStyle	Chromosome notation to convert to (default no conversion)
maxUTRbinSize	Max width of new alternative UTR bins
codingOnly	Logical, whether to keep only coding transcripts
genewise	Logical, whether annotation should be flattened genewise
stranded	Logical, whether to perform disjoin in a stranded fashion.
verbose	Logical, whether to print run information

Details

See the vignette for more details.

Value

A ‘GRanges‘ object.

Author(s)

Stefan Greber

Examples

```
data(example_gene_annotation)
bins <- prepareBins(example_gene_annotation)
```

---

rn6_PAS *Poly-A sites compendium for Rattus Norvegicus (Rno6)*

Description

These are the sites from polyA_DB release 3.2, downloaded from https://exon.apps.wistar.org/PolyA_DB/v3/download/3.2/rat_pas.zip, and lifted over to Rno6.

Value

a 'GRanges' object

---

simesAggregation *simesAggregation*

Description

Simes p-value correction and aggregation, adapted from `link[limma]{diffSplice}`

Usage

```
simesAggregation(p.value, geneid)
```

Arguments

p.value	A vector of p-values
geneid	A vector of group labels such as gene identifiers

Value

A named vector of aggregated p-values

Examples

```
p <- runif(50)
genes <- sample(LETTERS,50,replace=TRUE)
simesAggregation(p, genes)
```

Index

addNormalizedAssays, 2

countFeatures, *2*, 3, *4*, *7*, *9*, *11*

deuBinPlot, 4
DEUwrappers *(*diffSpliceDGEWrapper*)*, 6
DEXSeq, *6*
DEXSeqWrapper, *4*, *11*
DEXSeqWrapper *(*diffSpliceDGEWrapper*)*, 6
diffSplice, *5–7*
diffSplice2, 5, *7*
diffSpliceDGE, *6*
diffSpliceDGEWrapper, 6
diffSpliceWrapper, *4*, *11*
diffSpliceWrapper
        *(*diffSpliceDGEWrapper*)*, 6

example_bin_se, 7
example_gene_annotation, 8

geneBinHeatmap, 8
geneLevelStats, *7*, 9
geom_label_repel, *11*

Heatmap, *9*

lmFit, *5*

plotTopGenes, 11
prepareBins, 12

RangedSummarizedExperiment-class, *3*
rn6_PAS, 13

simesAggregation, 13
SingleAnnotation, *9*