

Package ‘diffUTR’

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Type Package

Title diffUTR: Streamlining differential exon and 3' UTR usage

Version 1.19.0

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

Suggests BiocStyle, knitr, rmarkdown

biocViews GeneExpression

BugReports <https://github.com/ETHZ-INS/diffUTR>

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| | |
|---------------------|----------------------------|
| addNormalizedAssays | <i>addNormalizedAssays</i> |
|---------------------|----------------------------|

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 | <i>countFeatures</i> |
|---------------|----------------------|

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 | <i>diffSplice2</i> |
|-------------|--------------------|

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","non-coding")' (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 | <i>geneLevelStats</i> |
|----------------|-----------------------|

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

| | |
|-----------------------------------|--|
| <code>se</code> | A ‘RangedSummarizedExperiment’ containing the results of one of the DEU wrappers. |
| <code>coef</code> | The coefficients tested (if the model included more than one term). |
| <code>excludeTypes</code> | Vector of bin types to exclude. |
| <code>includeTypes</code> | Vector of bin types to include (overrides ‘excludeTypes’) |
| <code>returnSE</code> | Logical; whether to return the updated ‘se’ object (default), or the gene-level table. |
| <code>minDensityRatio</code> | Minimum ratio of read density (with respect to the gene’s average) for a bin to be included. |
| <code>minWidth</code> | Minimum bin width to include |
| <code>excludeGeneAmbiguous</code> | 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 | <i>plotTopGenes</i> |
|--------------|---------------------|

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

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

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