Package ‘ballgown’

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Maintainer Jack Fu <jmfu@jhsph.edu>
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Description Tools for statistical analysis of assembled transcriptomes, including flexible differential expression analysis, visualization of transcript structures, and matching of assembled transcripts to annotation.
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Author Jack Fu [aut], Alyssa C. Frazee [aut, cre], Leonardo Collado-Torres [aut], Andrew E. Jaffe [aut], Jeffrey T. Leek [aut, ths]
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Description

Super awesome transcript-level expression analysis

annotate_assembly

match assembled transcripts to annotated transcripts

Description

match assembled transcripts to annotated transcripts

Usage

annotate_assembly(assembled, annotated)

Arguments

assembled GRangesList object representing assembled transcripts
annotated GRangesList object representing annotated transcripts

Details

If gown is a ballgown object, assembled can be structure(gown)$trans (or any subset). You can generate a GRangesList object containing annotated transcripts from a gtf file using the gffReadGR function and setting splitByTranscripts=TRUE.

Value

data frame, where each row contains assembledInd and annotatedInd (indexes of overlapping transcripts in assembled and annotated), and the percent overlap between the two transcripts.

Author(s)

Alyssa Frazee

Examples

data(bg)

gtfPath = system.file('extdata', 'annot.gtf.gz', package='ballgown')
annot = gffReadGR(gtfPath, splitByTranscript=TRUE)
info = annotate_assembly(assembled=structure(bg)$trans, annotated=annot)
Description

S4 class for storing and manipulating expression data from assembled transcriptomes

Slots

expr  tables containing expression data for genomic features (introns, exons, transcripts)
structure  genomic locations of features and their relationships to one another
indexes  tables connecting components of the assembly and providing other experimental information (e.g., phenotype data and locations of read alignment files)
dirs  directories holding data created by tablemaker
mergedDate  date the ballgown object was created
meas  which expression measurement(s) the object contains in its data slot. Vector of one or more of "rcount", "ucount", "mrcount", "cov", "cov_sd", "mcov", "mcov_sd", or "FPKM", if Tablemaker output is used, or one of "TPM" or "FPKM" if RSEM output is used. Can also be "all" for all measurements. See vignette for details.
RSEM  TRUE if object was made from RSEM output, FALSE if object was made from Tablemaker/Cufflinks output.

Author(s)

Alyssa Frazee, Leonardo Collado-Torres, Jeff Leek

Examples

data(bg)
class(bg) #"ballgown"
dim(bg@expr$exon)
bg@structure$exon
head(bg@indexes$t2g)
head(bg@dirs)
bg@mergedDate
bg@meas
bg@RSEM
ballgown-constructor

constructor function for ballgown objects

Description

constructor function for ballgown objects

Usage

ballgown(
  samples = NULL,
  dataDir = NULL,
  samplePattern = NULL,
  bamfiles = NULL,
  pData = NULL,
  verbose = TRUE,
  meas = "all"
)

Arguments

samples vector of file paths to folders containing sample-specific ballgown data (generated by tablemaker). If samples is provided, dataDir and samplePattern are not used.
dataDir file path to top-level directory containing sample-specific folders with ballgown data in them. Only used if samples is NULL.
samplePattern regular expression identifying the subdirectories of dataDir containing data to be loaded into the ballgown object (and only those subdirectories). Only used if samples is NULL.
bamfiles optional vector of file paths to read alignment files for each sample. If provided, make sure to sort properly (e.g., in the same order as samples). Default NULL.
pData optional data.frame with rows corresponding to samples and columns corresponding to phenotypic variables.
verbose if TRUE, print status messages and timing information as the object is constructed.
meas character vector containing either "all" or one or more of: "rcount", "ucount", "mrcount", "cov", "cov_sd", "mcov", "mcov_sd", or "FPKM". The resulting ballgown object will only contain the specified expression measurements, for the appropriate features. See vignette for which expression measurements are available for which features. "all" creates the full object.
Details

Because experimental data is recorded so variably, it is the user’s responsibility to format pData correctly. In particular, it’s really important that the rows of pData (corresponding to samples) are ordered the same way as samples or the dataDir/samplePattern combo. You can run list.files(path = dataDir, pattern = samplePattern) to see the sample order if samples was not used.

If you are creating a ballgown object for a large experiment, this function may run slowly and use a large amount of RAM. We recommend running this constructor as a batch job and saving the resulting ballgown object as an rda file. The rda file usually has reasonable size on disk, and the object in it shouldn’t take up too much RAM when loaded, so the time and memory use in creating the object is a one-time cost.

Value

an object of class ballgown

Author(s)

Leonardo Collado-Torres, Alyssa Frazee

See Also

ballgownrsem, for loading RSEM output into a ballgown object

Examples

bg = ballgown(dataDir=system.file('extdata', package='ballgown'),
               samplePattern='sample')
pData(bg) = data.frame(id=sampleNames(bg), group=rep(c(1,0), each=10))

ballgownrsem

load RSEM data into a ballgown object

Description

Loads results of rsem-calculate-expression into a ballgown object for easy visualization, processing, and statistical testing

Usage

ballgownrsem(
  dir = "",
  samples,
  gtf,
  UCSC = TRUE,
  tfield = "transcript_id",
  attrsep = "; ",
  bamout = "transcript",
  ...
Arguments

`dir` output directory containing RSEM output for all samples (i.e. for each run of rsem-calculate-expression)

`samples` vector of sample names (i.e., of the sample_name arguments used in each RSEM run)

`gtf` path to GTF file of genes/transcripts used in your RSEM reference. (where the reference location was denoted by the reference_name argument used in rsem-calculate-expression). RSEM references can be created with or without a GTF file, but currently the ballgown reader requires the GTF file.

`UCSC` set to TRUE if gtf comes from UCSC: quotes will be stripped from transcript identifiers if so.

`tfield` What keyword identifies transcripts in the "attributes" field of gtf? Default 'transcript_id'.

`attrsep` How are attributes separated in the "attributes" field of gtf? Default '; ' (semicolon-space).

`bamout` set to 'genome' if --output-genome-bam was used when running rsem-calculate-expression; set to 'none' if --no-bam-output was used when running rsem-calculate-expression; otherwise use the default ('transcript').

`pData` data frame of phenotype data, with rows corresponding to samples. The first column of pData must be equal to samples, and rows must be in the same order as samples.

`verbose` If TRUE (as by default), status messages are printed during data loading.

`meas` character vector containing either "all" or one of "FPKM" or "TPM". The resulting ballgown object will only contain the specified expression measurement for the transcripts. "all" creates the full object.

`zipped` set to TRUE if all RSEM results files have been gzipped (end) in ".gz").

Details

Currently exon- and intron-level measurements are not available for RSEM-generated ballgown objects, but development is ongoing.

Value

a ballgown object with the specified expression measurements and structure specified by GTF.

See Also

ballgown for reading Cufflinks/Tablemaker output
Examples

dataDir = system.file('extdata', package='ballgown')
gtf = file.path(dataDir, 'hg19_genes_small.gtf.gz')
rsemobj = ballgownrsem(dir=dataDir, samples=c('tiny', 'tiny2'), gtf=gtf,
  bamout='none', zipped=TRUE)
rsemobj

bg

Toy ballgown object

Description

Small ballgown object created with simulated toy data, for demonstration purposes

Format

a ballgown object: 100 transcripts, 633 exons, 536 introns

Author(s)

Alyssa Frazee

Examples

data(bg)
bg
# ballgown instance with 100 transcripts and 20 samples

checkAssembledTx

plot annotated and assembled transcripts together

Description

plot annotated and assembled transcripts together

Usage

checkAssembledTx(
  assembled,
  annotated,
  ind = 1,
  main = "Assembled and Annotated Transcripts",
  customCol = NULL
)


**Description**

group a gene’s assembled transcripts into clusters

**Usage**

```
clusterTranscripts(gene, gown, k = NULL, method = c("hclust", "kmeans"))
```
collapseTranscripts

collapseTranscripts

<table>
<thead>
<tr>
<th>Arguments</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>gene</td>
<td>name of gene whose transcripts will be clustered. When using Cufflinks output, usually of the form &quot;XLOC_#####&quot;</td>
</tr>
<tr>
<td>gown</td>
<td>ballgown object containing experimental data</td>
</tr>
<tr>
<td>k</td>
<td>number of clusters to use</td>
</tr>
<tr>
<td>method</td>
<td>clustering method to use. Must be one of &quot;hclust&quot;, for hierarchical clustering, or &quot;kmeans&quot;, for k-means clustering.</td>
</tr>
</tbody>
</table>

Value

list with elements clusters and pctvar. clusters contains columns "cluster" and "t_id", and denotes which transcripts belong to which clusters. pctvar is only non-NULL when using k-means clustering and is the percentage of variation explained by these clusters, defined as the ratio of the between-cluster sum of squares to the total sum of squares.

Author(s)

Alyssa Frazee

See Also

hclust, kmeans, plotLatentTranscripts for visualizing the transcript clusters

Examples

data(bg)
clusterTranscripts('XLOC_000454', bg, k=2, method='kmeans')
# transcripts 1294 and 1301 cluster together, 91% variation explained.

collapseTranscripts | cluster a gene’s transcripts and calculate cluster-level expression

description

collapseTranscripts

collapseTranscripts(gene, gown, meas = "FPKM", method = c("hclust", "kmeans"), k = NULL)
**contains**

**Arguments**

- **gene**: which gene’s transcripts should be clustered
- **gown**: ballgown object
- **meas**: which transcript-level expression measurement to use (‘cov’, average per-base coverage, or ‘FPKM’)
- **method**: which clustering method to use: ‘hclust’ (hierarchical clustering) or ‘kmeans’ (k-means clustering).
- **k**: how many clusters to use.

**Value**

List with two elements:

- **tab**: a cluster-by-sample table of expression measurements (meas, either cov or FPKM), where the expression measurement for each cluster is the mean (for ‘cov’) or aggregate (for ‘FPKM’, as in gexpr) expression measurement for all the transcripts in that cluster. This table can be used as the gowntable argument to stattest, if differential expression results for transcript *clusters* are desired.
- **cl**: output from clusterTranscripts that was run to produce tab, for reference. Cluster IDs in the cluster component correspond to row names of tab

**Author(s)**

Alyssa Frazee

**See Also**

hclust, kmeans, clusterTranscripts, plotLatentTranscripts

**Examples**

data(bg)
collapseTranscripts(bg, gene='XLOC_000454', meas='FPKM', method='kmeans')

**Description**

determine if one set of GRanges fully contains any of another set of GRanges

**Usage**

contains(transcripts, cds)
Arguments

transcripts  GRangesList object (assume for now that it represents transcripts)
cds         GRangesList object (assume for now that it represents coding sequences)

Details

If `gown` is a ballgown object, `transcripts` can be `structure(gown)$trans` (or any subset).

Value

vector with length equal to `length(transcripts)`, where each entry is TRUE if the corresponding transcript contains a coding sequence (i.e., is a superset of at least one entry of `cds`).

Author(s)

Alyssa Frazee

Examples

```r
## pretend this annotation is coding sequence:
gtfPath = system.file('extdata', 'annot.gtf.gz', package='ballgown')
annot = gffReadGR(gtfPath, splitByTranscript=TRUE)
data(bg)
results = contains(structure(bg)$trans, annot)
# results is a boolean vector
sum(results) #61
```

`dirs`  extract paths to tablemaker output

Description

extract paths to tablemaker output

Usage

dirs(x)

```r
## S4 method for signature 'ballgown'
dirs(x)
```

Arguments

x  a ballgown object

Examples

data(bg)
dirs(bg)
**eexpr**

*extract exon-level expression measurements from ballgown objects*

**Description**

extract exon-level expression measurements from ballgown objects

**Usage**

```r
eexpr(x, meas = "rcount")
```

### S4 method for signature 'ballgown'

```r
eexpr(x, meas = "rcount")
```

**Arguments**

- **x**
  - a ballgown object
- **meas**
  - type of measurement to extract. Can be "rcount", "ucount", "mrcount", "cov", "mcov", or "all". Default "rcount".

**Value**

exon-by-sample matrix containing exon-level expression values (measured by `meas`). If `meas` is "all", or `x@RSEM` is TRUE, a data frame is returned, containing all measurements and location information.

**Examples**

```r
data(bg)
exon_rcount_matrix = eexpr(bg)
exon_ucount_matrix = eexpr(bg, 'ucount')
exon_data_frame = eexpr(bg, 'all')
```

**expr**

*extract expression components from ballgown objects*

**Description**

extract expression components from ballgown objects

**Usage**

```r
expr(x)
```

### S4 method for signature 'ballgown'

```r
expr(x)
```
Arguments

x a ballgown object

Value

list containing elements intron, exon, and trans, which are feature-by-sample data frames of expression data.

See Also
texpr, gexpr, eexpr, iexpr

Examples

data(bg)
names(expr(bg))
class(expr(bg))
dim(expr(bg)$exon)

data(bg)
n = ncol(bg@expr$trans)
#multiply all transcript expression measurements by 10:
bg@expr$trans[,1:n] = 10*bg@expr$trans[11:n]

expr<-

Replacement method for expr slot in ballgown objects

Description

Replacement method for expr slot in ballgown objects

Usage

expr(x) <- value

## S4 replacement method for signature 'ballgown'
expr(x) <- value

Arguments

x a ballgown object

value the updated value for expr(x) or a subcomponent

Examples

data(bg)
n = ncol(bg@expr$trans)
#multiply all transcript expression measurements by 10:
bg@expr$trans[,1:n] = 10*bg@expr$trans[11:n]
exprfilter  

subset ballgown objects using an expression filter

Description

Create a new ballgown object containing only transcripts passing a mean expression filter

Usage

exprfilter(gown, cutoff, meas = "FPKM")

Arguments

gown  
a ballgown object

cutoff  
transcripts must have mean expression across samples above this value to be included in the return

meas  
how should transcript expression be measured? Default FPKM, but can also be 'cov'.

Value

A new ballgown object derived from gown, but only containing transcripts (and associated exons/introns) with mean meas greater than cutoff across all samples.

See Also

subset

Examples

data(bg)

# make a ballgown object containing only transcripts with mean FPKM > 100:

over100 = exprfilter(bg, cutoff=100)

geneIDs  
get gene IDs from a ballgown object

Description

gene IDs from a ballgown object
Usage

geneIDs(x)

## S4 method for signature 'ballgown'
geneIDs(x)

Arguments

x a ballgown object

Details

This vector differs from that produced by geneNames in that geneIDs produces names of loci created during the assembly process, not necessarily annotated genes.

Value

named vector of gene IDs included in the ballgown object. If object was created using Tablemaker, these gene IDs will be of the form "XLOC_+". Vector is named and ordered by corresponding numeric transcript ID.

See Also

geneNames

data(bg)
geneIDs(bg)

geneNames get gene names from a ballgown object

description

geneNames get gene names from a ballgown object

Usage

geneNames(x)

## S4 method for signature 'ballgown'
geneNames(x)

Arguments

x a ballgown object
**getAttributeField**

extract a specific field of the "attributes" column of a data frame created from a GTF/GFF file

Description

extract a specific field of the "attributes" column of a data frame created from a GTF/GFF file

Usage

getAttributeField(x, field, attrsep = ";")

Arguments

- **x**: vector representing the "attributes" column of GTF/GFF file
- **field**: name of the field you want to extract from the "attributes" column
- **attrsep**: separator for the fields in the attributes column. Defaults to '; ', the separator for GTF files outputted by Cufflinks.
Value

vector of nucleotide positions included in the transcript

Author(s)

Wolfgang Huber, in the davidTiling R package (LGPL license)

See Also

gffRead for creating a data frame from a GTF/GFF file, and http://useast.ensembl.org/info/website/upload/gff.html for specifics of the GFF/GTF file format.

Examples

```r
gtfPath = system.file('extdata', 'annot.gtf.gz', package='ballgown')
gffdata = gffRead(gtfPath)
gffdata$transcriptID = getAttributeField(gffdata$attributes, field = "transcript_id")
```

---

getGenes

**label assembled transcripts with gene names**

Description

label assembled transcripts with gene names

Usage

```r
getGenes(gtf, assembled, UCSC = TRUE, attribute = "gene_id")
```

Arguments

- **gtf**
  - path to a GTF file containing locations of annotated transcripts

- **assembled**
  - GRangesList object, with each set of ranges representing exons of an assembled transcript.

- **UCSC**
  - set to TRUE if you’re using a UCSC gtf file. (Requires some extra text processing).

- **attribute**
  - set to attribute name in gtf that gives desired gene identifiers. Default "gene_id"; another common one is "gene_name" (for the gene symbol).

Details

chromosome labels in gtf and assembled should match. (i.e., you should provide the path to a gtf corrsponding to the same annotation you used when constructing assembled)
gexpr

Value

an IRanges CharacterList of the same length as assembled, providing the name(s) of the gene(s) that overlaps each transcript in assembled.

Author(s)

Alyssa Frazee, Andrew Jaffe

Examples

data(bg)  
gtfPath = system.file('extdata', 'annot.gtf.gz', package='ballgown')  
geneoverlaps = getGenes(gtfPath, structure(bg)$trans, UCSC=FALSE)

gexpr

extract gene-level expression measurements from ballgown objects

Description

For objects created with Cufflinks/Tablemaker, gene-level measurements are calculated by appropriately combining FPKMs from the transcripts comprising the gene. For objects created with RSEM, gene-level measurements are extracted directly from the RSEM output.

Usage

gexpr(x)

## S4 method for signature 'ballgown'
gexpr(x)

Arguments

x a ballgown object

Value

gene-by-sample matrix containing per-sample gene measurements.

Examples

data(bg)  
gene_matrix = gexpr(bg)
gffRead

read in GTF/GFF file as a data frame

Description

read in GTF/GFF file as a data frame

Usage

gffRead(gffFile, nrows = -1, verbose = FALSE)

Arguments

- `gffFile`: name of GTF/GFF on disk
- `nrows`: number of rows to read in (default -1, which means read all rows)
- `verbose`: if TRUE, print status info at beginning and end of file read. Default FALSE.

Value

data frame representing the GTF/GFF file

Author(s)

Kasper Hansen

See Also

getAttributeField to extract data from "attributes" column; [http://useast.ensembl.org/info/website/upload/gff.html](http://useast.ensembl.org/info/website/upload/gff.html) for more information on the GTF/GFF file format.

Examples

```r
gtfPath = system.file('extdata', 'annot.gtf.gz', package='ballgown')
anot = gffRead(gtfPath)
```

gffReadGR

read in gtf file as GRanges object

Description

(very) light wrapper for rtracklayer::import
gffReadGR

Usage

gffReadGR(
  gtf,
  splitByTranscript = FALSE,
  identifier = "transcript_id",
  sep = "; ")

Arguments

  gtf          name of GTF/GFF file on disk
  splitByTranscript
                 if TRUE, return a GRangesList of transcripts; otherwise return a GRanges object
                 containing all genomic features in gtf. Default FALSE.
  identifier   name of transcript identifier column of attributes field in gtf. Default "transcript_id".
                 Only used if splitByTranscript is TRUE.
  sep          field separator in the attributes field of gtf. Default "; " (semicolon + space).
                 Only used if splitByTranscript is TRUE.

Value

  if splitByTranscript is FALSE, an object of class GRanges representing the genomic features in gtf. If splitByTranscript is TRUE, an object of class GRangesList, where each element is a GRanges object corresponding to an annotated transcript (designated in names).

Author(s)

  Alyssa Frazee

See Also

  gffRead for reading in a GTF file as a data frame rather than a GRanges/GRangesList object.

Examples

  gtfPath = system.file('extdata', 'annot.gtf.gz', package='ballgown')

  # read in exons as GRanges:
  annotgr = gffReadGR(gtfPath)

  # read in groups of exons as transcripts, in GRangesList:
  transcripts_grl = gffReadGR(gtfPath, splitByTranscript=TRUE)
iexpr

extract transcript-level expression measurements from ballgown objects

Description

extract transcript-level expression measurements from ballgown objects

Usage

iexpr(x, meas = "rcount")

## S4 method for signature 'ballgown'
iexpr(x, meas = "rcount")

Arguments

x

a ballgown object

meas
type of measurement to extract. Can be "rcount", "ucount", "mrcount", or "all". Default "rcount".

Value

intron-by-sample matrix containing the number of reads (measured as specified by meas) supporting each intron, in each sample. If meas is "all", a data frame is returned, containing all measurements and location information.

Examples

data(bg)
intron_rcount_matrix = iexpr(bg)
intron_data_frame = iexpr(bg, 'all')

indexes

extract the indexes from ballgown objects

Description

extract the indexes from ballgown objects

Usage

indexes(x)

## S4 method for signature 'ballgown'
indexes(x)

## S4 method for signature 'ballgown'
indexes(x)
indexes<-  

Arguments  

x a ballgown object  

Value  

list containing elements e2t, i2t, t2g, bamfiles, and pData, where e2t and i2t are data frames linking exons and introns (respectively) to transcripts. t2g is a data frame linking transcripts to genes, and bamfiles and pData are described in ?ballgown.  

Examples  

data(bg)  
names(indexes(bg))  
class(indexes(bg))  
head(indexes(bg)$t2g)  

indexes<- Replace method for indexes slot in ballgown objects  

Description  

Replace method for indexes slot in ballgown objects  

Usage  

indexes(x) <- value  

## S4 replacement method for signature 'ballgown'  
indexes(x) <- value  

Arguments  

x a ballgown object  

value the updated value for indexes(x) or a subcomponent  

Examples  

data(bg)  
indexes(bg)$bamfiles = paste0('/path/to/bamfolder/',  
sampleNames(bg), '_accepted_hits.bam')
### last

*get the last element*

### Description

get the last element

### Usage

```r
last(x)
```

### Arguments

- `x`: anything you can call `tail` on (vector, data frame, etc.)

### Details

this function is made of several thousand lines of complex code, so be sure to read it carefully.

### Value

the last element of `x`

### Author(s)

Alyssa Frazee

### Examples

```r
last(c('h', 'e', 'l', 'l', 'o'))
```

---

### mergedDate

*extract package version & creation date from ballgown object*

### Description

extract package version & creation date from ballgown object

### Usage

```r
mergedDate(x)
```

```r
## S4 method for signature 'ballgown'
mergedDate(x)
```
**pctOverlap**

**Arguments**

\(x\)  
a ballgown object

**Examples**

```r
data(bg)
mergedDate(bg)
```

**pctOverlap**  
*calculate percent overlap between two GRanges objects*

**Description**

calculate percent overlap between two GRanges objects

**Usage**

\(\text{pctOverlap}(\text{tx1}, \text{tx2})\)

**Arguments**

\(\text{tx1}\)  
GRanges object

\(\text{tx2}\)  
GRanges object

**Details**

In the ballgown context, \(\text{tx1}\) and \(\text{tx2}\) are two transcripts, each represented by GRanges objects whose ranges represent the exons comprising the transcripts. The percent overlap is the number of nucleotides falling within both transcripts divided by the number of nucleotides falling within either transcript. Useful as a measure of transcript closeness (as it is essentially Jaccard distance).

**Value**

percent overlap between \(\text{tx1}\) and \(\text{tx2}\), as defined by the ratio of the intersection of \(\text{tx1}\) and \(\text{tx2}\) to the union of \(\text{tx1}\) and \(\text{tx2}\).

**Author(s)**

Alyssa Frazee

**Examples**

```r
data(bg)

gtfPath = system.file('extdata', 'annot.gtf.gz', package='ballgown')
anno_grl = gffReadGR(gtfPath, splitByTranscript=TRUE)
pctOverlap(structure(bg)$trans[[2]], anno_grl[[369]]) #79.9%
```
pData <- replacement method for pData slot in ballgown objects

Description
Replacement method for pData slot in ballgown objects

Usage
pData(object) <- value
  ## S4 replacement method for signature 'ballgown,ANY'
    pData(object) <- value

Arguments
  object a ballgown object
  value the updated value for pData(x).

pData <-

Description
extract phenotype data from a ballgown object

Usage
pData(object)

Arguments
  object a ballgown object

Value
sample-by-phenotype data frame

Examples
data(bg)
pData(bg)

pData <-

Description
extract phenotype data from a ballgown object

Usage
pData(object)

Arguments
  object a ballgown object

Value
sample-by-phenotype data frame

Examples
data(bg)
pData(bg)
plotLatentTranscripts

Examples

   # add "timepoint" covariate to ballgown object:
   data(bg) # already contains pData
   pData(bg) = data.frame(pData(bg), timepoint=rep(1:10, 2))
   head(pData(bg))

plotLatentTranscripts  cluster assembled transcripts and plot the results

Description

This is an experimental, first-pass function that clusters assembled transcripts based on their overlap percentage, then plots and colors the transcript clusters.

Usage

plotLatentTranscripts(  
gene,  
gown,  
method = c("hclust", "kmeans"),  
k = NULL,  
choosek = c("var90", "thumb"),  
returncluster = TRUE,
  labelTranscripts = TRUE,
  ...
)

Arguments

gene  
string, name of gene whose transcripts should be clustered (e.g., "XLOC_000001")
gown  
object of class ballgown being used for analysis
method  
clustering method to use. Currently can choose from hierarchical clustering (hclust) or K-means (kmeans). More methods are in development.
k  
count of transcript clusters to use. By default, k is NULL and thus is chosen using a rule of thumb, but providing k overrides those rules of thumb.
choosek  
if k is not provided, how should the number of clusters be chosen? Must be one of "var90" (choose a k that explains 90 percent of the observed variation) or "thumb" (k is set to be approximately sqrt(n), where n is the total number of transcripts for gene)
returncluster  
if TRUE (as it is by default), return the results of the call to clusterTrancsripts so the data is available for later use. Nothing is returned if FALSE.
labelTranscripts  
if TRUE (as it is by default), print transcript IDs on the y-axis
...  
other arguments to pass to plotTranscripts
plotMeans

Value

If `returnCluster` is TRUE, the transcript clusters are returned as described in `clusterTranscripts`. A plot of the transcript clusters is also produced, in the style of `plotTranscripts`.

Author(s)

Alyssa Frazee

See Also

`clusterTranscripts, plotTranscripts`

Examples

data(bg)
plotLatentTranscripts('XLOC_000454', bg, method='kmeans', k=2)

---

**plotMeans**  visualize transcript abundance by group

**Description**

visualize transcript abundance by group

**Usage**

plotMeans(
  gene,
  gown,
  overall = FALSE,
  groupvar,
  groupname = "all",
  meas = c("cov", "FPKM", "rcount", "ucount", "mrcount", "mcov"),
  colorby = c("transcript", "exon"),
  legend = TRUE,
  labelTranscripts = FALSE
)

**Arguments**

- **gene**: name of gene whose transcripts will be plotted. When using Cufflinks/Tablemaker output, usually of the form "XLOC_#####"
- **gown**: ballgown object containing experimental and phenotype data
- **overall**: if TRUE, color features by the overall (experiment-wide) mean rather than a group-specific mean
plotTranscripts

**Groupvar**

string representing the name of the variable denoting which sample belongs to which group. Can be "none" (if you want the study-wide mean), or must correspond to the name of a column of pData(gown). Usually a categorical variable.

**Groupname**

string representing which group’s expression means you want to plot. Can be "none" (if you want the study-wide mean), “all” (if you want a multipanel plot of each group’s mean expression), or any of the levels of groupvar.

**Meas**

type of expression measurement to plot. One of "cov", "FPKM", "rcount", "ucount", "mrcount", or "mcov". Not all types are valid for all features. (See description of tablemaker output for more information).

**Colorby**

one of "transcript" or "exon", indicating which feature’s abundances should dictate plot coloring.

**Legend**

if TRUE (as it is by default), a color legend is drawn on top of the plot indicating the scale for feature abundances.

**LabelTranscripts**

if TRUE, transcript ids are labeled on the left side of the plot. Default FALSE.

**Value**

produces a plot of the transcript structure for the specified gene in the current graphics device, colored by study-wide or group-specific mean expression level.

**Author(s)**

Alyssa Frazee

**See Also**

plotTranscripts

**Examples**

data(bg)
plotMeans('XLOC_000454', bg, groupvar='group', meas='FPKM', colorby='transcript')

---

**plotTranscripts**

visualize structure of assembled transcripts

**Description**

visualize structure of assembled transcripts
plotTranscripts(
  gene,
  gown,
  samples = NULL,
  colorby = "transcript",
  meas = "FPKM",
  legend = TRUE,
  labelTranscripts = FALSE,
  main = NULL,
  blackBorders = TRUE,
  log = FALSE,
  logbase = 2,
  customCol = NULL,
  customOrder = NULL
)

Arguments

gene name of gene whose transcripts will be plotted. When using Cufflinks output, usually of the form "XLOC_#####"
gown ballgown object containing experimental and phenotype data
samples vector of sample(s) to plot. Can be 'none' if only one plot (showing transcript structure in gray) is desired. Use sampleNames(gown) to see sample names for gown. Defaults to sampleNames(gown)[1].
colorby one of "transcript", "exon", or "none", indicating which feature's abundances should dictate plot coloring. If "none", all transcripts are drawn in gray.
meas which expression measurement to color features by, if any. Must match an available measurement for whatever feature you're plotting.
legend if TRUE (as it is by default), a color legend is drawn on top of the plot indicating scales for feature abundances.
labelTranscripts if TRUE, transcript ids are labeled on the left side of the plot. Default FALSE.
maint optional string giving the desired plot title.
blackBorders if TRUE, exon borders are drawn in black. Otherwise, they are drawn in the same color as their transcript or exon. Switching blackBorders to FALSE can be useful for visualizing abundances for skinny exons and/or smaller plots, which can be the case when length(samples) is large.
log if TRUE, color transcripts on the log scale. Default FALSE. To account for expression values of 0, we add 1 to all expression values before taking the log.
logbase log base to use if log = TRUE. Default 2.
customCol an optional vector of custom colors to color transcripts by. There must be the same number of colors as transcripts in the gene being plotted.
customOrder an optional vector of transcript ids (matching ids in texpr(gown, 'all')$t_id), indicating which order transcripts will appear in the plot. All transcripts in gene must appear in the vector exactly once.
*sampleNames*

**Value**

produces a plot of the transcript structure for the specified gene in the current graphics device.

**Author(s)**

Alyssa Frazee

**See Also**

`plotMeans`, `plotLatentTranscripts`

**Examples**

```r
data(bg)

# plot one gene for one sample:
plotTranscripts(gene='XLOC_000454', gown=bg, samples='sample12', meas='FPKM',
               colorby='transcript',
               main='transcripts from gene XLOC_000454: sample 12, FPKM')

# plot one gene for many samples:
plotTranscripts('XLOC_000454', bg,
                samples=c('sample01', 'sample06', 'sample12', 'sample19'),
                meas='FPKM', colorby='transcript')
```

---

**sampleNames**

get names of samples in a ballgown objects

**Description**

get names of samples in a ballgown objects

**Usage**

```r
sampleNames(object)
```

## S4 method for signature 'ballgown'

```r
sampleNames(object)
```

**Arguments**

- `object` a ballgown object

**Value**

vector of sample IDs for `x`. If `pData` exists, samples in its rows correspond to samples in `sampleNames(x)` (in order).
Examples

```r
data(bg)
sampleNames(bg)
```

---

**seqnames**

*get sequence (chromosome) names from ballgown object*

---

**Description**

get sequence (chromosome) names from ballgown object

**Usage**

```r
seqnames(x)
```

```r
## S4 method for signature 'ballgown'
seqnames(x)
```

**Arguments**

x

a ballgown object

**Value**

vector of sequence (i.e., chromosome) names included in the ballgown object

**Examples**

```r
data(bg)
seqnames(bg)
```

---

**stattest**

*statistical tests for differential expression in ballgown*

---

**Description**

Test each transcript, gene, exon, or intron in a ballgown object for differential expression, using comparisons of linear models.
Usage

stattest(
  gown = NULL,
  gowntable = NULL,
  pData = NULL,
  mod = NULL,
  mod0 = NULL,
  feature = c("gene", "exon", "intron", "transcript"),
  meas = c("cov", "FPKM", "rcount", "ucount", "mrcount", "mcov"),
  timecourse = FALSE,
  covariate = NULL,
  adjustvars = NULL,
  gexpr = NULL,
  df = 4,
  getFC = FALSE,
  libadjust = NULL,
  log = TRUE
)

Arguments

gown name of an object of class ballgown
gowntable matrix or matrix-like object with rownames representing feature IDs and columns representing samples, with expression estimates in the cells. Provide the feature name with feature. You must provide exactly one of gown or gowntable. NB: gowntable is log-transformed within stattest if log is TRUE, so provide unlogged expression values in gowntable.
pData Required if gowntable is provided: data frame giving phenotype data for the samples in the columns of gowntable. (Rows of pData correspond to columns of gowntable). If gown is used instead, it must have a non-null, valid pData slot (and the pData argument to stattest should be left NULL).
mod object of class model.matrix representing the design matrix for the linear regression model including covariates of interest
mod0 object of class model.matrix representing the design matrix for the linear regression model without the covariates of interest.
feature the type of genomic feature to be tested for differential expression. If gown is used, must be one of "gene", "transcript", "exon", or "intron". If gowntable is used, this is just used for labeling and can be whatever the rows of gowntable represent.
meas the expression measurement to use for statistical tests. Must be one of "cov", "FPKM", "rcount", "ucount", "mrcount", or "mcov". Not all expression measurements are available for all features. Leave as default if gowntable is provided.
timecourse if TRUE, tests whether or not the expression profiles of genomic features vary over time (or another continuous covariate) in the study. Default FALSE. Natural splines are used to fit time profiles, so you must have more timepoints than degrees of freedom used to fit the splines. The default df is 4.
covariate
string representing the name of the covariate of interest for the differential expression tests. Must correspond to the name of a column of pData(gown). If timecourse=TRUE, this should be the study's time variable.

adjustvars
optional vector of strings representing the names of potential confounders. Must correspond to names of columns of pData(gown).

gexpr
optional data frame that is the result of calling gexpr(gown)). (You can speed this function up by pre-creating gexpr(gown).)

df
degrees of freedom used for modeling expression over time with natural cubic splines. Default 4. Only used if timecourse=TRUE.

getFC
if TRUE, also return estimated fold changes (adjusted for library size and confounders) between populations. Only available for 2-group comparisons at the moment. Default FALSE.

libadjust
library-size adjustment to use in linear models. By default, the adjustment is defined as the sum of the sample’s log expression measurements below the 75th percentile of those measurements. To use a different library-size adjustment, provide a numeric vector of each sample’s adjustment value. Entries of this vector correspond to samples in in rows of pData. If no library size adjustment is desired, set to FALSE.

log
if TRUE, outcome variable in linear models is log(expression+1), otherwise it’s expression. Default TRUE.

Details
At minimum, you need to provide a ballgown object or count table, the type of feature you want to test (gene, transcript, exon, or intron), the expression measurement you want to use (FPKM, cov, rcount, etc.), and the covariate of interest, which must be the name of one of the columns of the ‘pData’ component of your ballgown object (or provided pData). This covariate is automatically converted to a factor during model fitting in non-timecourse experiments.

By default, models are fit using log2(meas + 1) as the outcome for each feature. To disable the log transformation, provide ‘log = FALSE’ as an argument to ‘stattest’. You can use the gowntable option if you’d like to to use a different transformation.

Library size adjustment is performed by default by using the sum of the log nonzero expression measurements for each sample, up to the 75th percentile of those measurements. This adjustment can be disabled by setting libadjust=FALSE. You can use mod and mod0 to specify alternative library size adjustments.

mod and mod0 are optional arguments. If mod is specified, you must also specify mod0. If neither is specified, mod0 defaults to the design matrix for a model including only a library-size adjustment, and mod defaults to the design matrix for a model including a library-size adjustment and covariate. Note that if you supply mod and mod0, covariate, timecourse, adjustvars, and df are ignored, so make sure your covariate of interest and all appropriate confounder adjustments, including library size, are specified in mod and mod0. By default, the library-size adjustment is the sum of all counts below the 75th percentile of nonzero counts, on the log scale (log2 + 1).

Full model details are described in the supplement of http://biorxiv.org/content/early/2014/03/30/003665.
Value

data frame containing the columns feature, id representing feature id, pval representing the p-value for testing whether this feature was differentially expressed according to covariate, and qval, the estimated false discovery rate using this feature’s signal strength as a significance cutoff. An additional column, fc, is included if getFC is TRUE.

Author(s)

Jeff Leek, Alyssa Frazee

References

http://biorxiv.org/content/early/2014/03/30/003665

Examples

data(bg)

# two-group comparison:
stat_results = stattest(bg, feature='transcript', meas='FPKM',
              covariate='group')

# timecourse test:
pData(bg) = data.frame(pData(bg), time=rep(1:10, 2)) #dummy time covariate
timecourse_results = stattest(bg, feature='transcript', meas='FPKM',
                           covariate='time', timecourse=TRUE)

# timecourse test, adjusting for group:
group_adj_timecourse_results = stattest(bg, feature='transcript',
                           meas='FPKM', covariate='time', timecourse=TRUE, adjustvars='group')

# custom model matrices:
### create example data:
set.seed(43)
sex = sample(c('M','F'), size=nrow(pData(bg)), replace=TRUE)
age = sample(21:52, size=nrow(pData(bg)), replace=TRUE)

### create design matrices:
mod = model.matrix(~ sex + age + pData(bg)$group + pData(bg)$time)
mod0 = model.matrix(~ pData(bg)$group + pData(bg)$time)

### build model:
adjusted_results = stattest(bg, feature='transcript', meas='FPKM',
                   mod0=mod0, mod=mod)
**structure**

*extract structure components from ballgown objects*

**Description**

extract structure components from ballgown objects

**Usage**

```r
structure(x)
```

## S4 method for signature 'ballgown'
structure(x)

**Arguments**

- `x`  
a ballgown object

**Value**

list containing elements intron, exon, and trans. exon and intron are GRanges objects, where each range is an exon or intron, and trans is a GRangesList object, where each GRanges element is a set of exons representing a transcript.

**Examples**

```r
data(bg)
names(structure(bg))
class(structure(bg))
structure(bg)$exon
```

---

**subset**

*subset ballgown objects to specific samples or genomic locations*

**Description**

subset ballgown objects to specific samples or genomic locations

**Usage**

```r
subset(x, ...)
```

## S4 method for signature 'ballgown'
subset(x, cond, genomesubset = TRUE)

**Arguments**

- `x`  
a ballgown object
- `cond`  
  condition for subset
- `genomesubset`  
  logical, if TRUE, subset ballgown objects to specific genomic locations

**Value**

subset ballgown objects to specific samples or genomic locations
**Arguments**

- **x**: a ballgown object
- ... further arguments to generic subset
- **cond**: Condition on which to subset. See details.
- **genomesubset**: if TRUE, subset x to a specific part of the genome. Otherwise, subset x to only include specific samples. TRUE by default.

**Details**

To use subset, you must provide the `cond` argument as a string representing a logical expression specifying your desired subset. The subset expression can either involve column names of `texpr(x, "all")` (if genomesubset is TRUE) or of `pData(x)` (if genomesubset is FALSE). For example, if you wanted a ballgown object for only chromosome 22, you might call `subset(x, "chr == 'chr22'")`. (Be sure to handle quotes within character strings appropriately).

**Value**

a subsetted ballgown object, containing only the regions or samples satisfying `cond`.

**Author(s)**

Alyssa Frazee

**Examples**

```r
data(bg)
bgs = subset(bg, "gene_id=='XLOC_000454' | gene_id=='XLOC_000024'")
bgs # ballgown instance with 4 assembled transcripts and 20 samples
```

```r
bg_group0 = subset(bg, "group == 0", genomesubset=FALSE)
bg_group0 # ballgown instance with 100 assembled transcripts and 10 samples
```

---

**texpr**

extract transcript-level expression measurements from ballgown objects

**Description**

extract transcript-level expression measurements from ballgown objects

**Usage**

```r
texpr(x, meas = "FPKM")
```

```r
# S4 method for signature 'ballgown'
texpr(x, meas = "FPKM")
```
**tGene**

**Arguments**

- **x**: a ballgown object
- **meas**: type of measurement to extract. Can be "cov", "FPKM", or "all". Default "FPKM".

**Value**

transcript-by-sample matrix containing expression values (measured by meas). If meas is "all", a data frame is returned, containing all measurements and location information.

**Examples**

```r
data(bg)
transcript_fpkm_matrix = texpr(bg)
transcript_data_frame = texpr(bg, 'all')
```

---

**tGene**

*Connect a transcript to its gene*

**Description**

find the gene to which a transcript belongs

**Usage**

`tGene(bg, transcript, tid = TRUE, gid = TRUE, warnme = TRUE)`

**Arguments**

- **bg**: ballgown object
- **transcript**: transcript identifier
- **tid**: set to TRUE if transcript is a numeric transcript identifier (i.e., t_id in expression tables), or FALSE if transcript is a named identifier (e.g., TCONS_000001 or similar.
- **gid**: if FALSE, return the gene *name* associated with transcript in bg instead of the gene *id*, which is returned by default. Take care to remember that not all ballgown objects include gene *name* information. (They do all include gene IDs).
- **warnme**: if TRUE, and if gid is FALSE, print a warning if no gene name is available for the transcript. This could either mean the transcript didn’t overlap an annotated gene, or that no gene names were included when bg was created.
transcriptIDs

Examples

data(bg)
tGene(bg, 10)
tGene(bg, 'TCONS_00000010', tid=FALSE)
tGene(bg, 10, gid=FALSE) # empty: no gene names included in bg.

Description

get numeric transcript IDs from a ballgown object

Usage

transcriptIDs(x)

## S4 method for signature 'ballgown'
transcriptIDs(x)

Arguments

x a ballgown object

Value

vector of numeric transcript IDs included in the ballgown object

Examples

data(bg)
transcriptIDs(bg)

transcriptNames

get transcript names from a ballgown object

Description

get transcript names from a ballgown object

Usage

transcriptNames(x)

## S4 method for signature 'ballgown'
transcriptNames(x)
writeFiles

Arguments

x a ballgown object

Value

vector of transcript names included in the ballgown object. If object was created using Cuff-
flinks/Tablemaker, these transcript names will be of the form "TCONS_*". Return vector is named
and ordered by corresponding numeric transcript ID.

Examples

data(bg)
transcriptNames(bg)

writeFiles write files to disk from ballgown object

Description

create tablemaker-like files on disk from a ballgown object

Usage

writeFiles(gown, dataDir)

Arguments

gown ballgown object
dataDir top-level directory for sample-specific folders

Examples

data(bg)
writeFiles(bg, dataDir=getwd())
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