Package ‘cummeRbund’

December 21, 2016

Title Analysis, exploration, manipulation, and visualization of Cufflinks high-throughput sequencing data.

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Author L. Goff, C. Trapnell, D. Kelley

Description Allows for persistent storage, access, exploration, and manipulation of Cufflinks high-throughput sequencing data. In addition, provides numerous plotting functions for commonly used visualizations.

Imports methods, plyr, BiocGenerics, S4Vectors (>= 0.9.25), Biobase

Depends R (>= 2.7.0), BiocGenerics (>= 0.3.2), RSQLite, ggplot2, reshape2, fastcluster, rtracklayer, Gviz

Suggests cluster, plyr, NMFN, stringr, GenomicFeatures, GenomicRanges, rjson

Maintainer Loyal A. Goff <lgoff@csail.mit.edu>

License Artistic-2.0


LazyLoad yes

biocViews HighThroughputSequencing, HighThroughputSequencingData, RNAseq, RNAseqData, GeneExpression, DifferentialExpression, Infrastructure, DataImport, DataRepresentation, Visualization, Bioinformatics, Clustering, MultipleComparisons, QualityControl

NeedsCompilation no

R topics documented:

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cummeRbund-package

Description

Allows for persistent storage, access, and manipulation of Cufflinks high-throughput sequencing data. In addition, provides numerous plotting functions for commonly used visualizations.

Details

Package: cummeRbund
Version: 0.1.3
Suggests:
Depends: R (>= 2.7.0), RSQLite, reshape2, ggplot2, methods
License: MIT License
LazyLoad: yes
biocViews: HighThroughputSequencing, HighThroughputSequencingData, RNAseq, RNAseqData, GeneExpression, DataImport, DataRepresentation, Visualization, Bioinformatics, Clustering, MultipleComparisons, QualityControl
Packaged: 2011-08-05 18:03:50 UTC; lgoff
Built: R 2.12.1; 2011-08-05 18:03:57 UTC; unix

CuffData-class
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CuffFeature-class
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CuffGene-class
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addFeatures
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createDB
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csDensity-methods
csHeatmap
csHeatmap-methods
csScatter
cummeRbund-package

csScatter-methods ~~ Methods for Function csScatter in Package 'cummeRbund' ~~
csVolcano Volcano Plot

csVolcano-methods ~~ Methods for Function csVolcano in Package 'cummeRbund' ~~
cummeRbund-package cummeRbund: The finishing touch on your Tuxedo workflow. Analysis, manipulation, and visualization of Cufflinks HTS data.
diffData Differential comparison data
diffData-methods ~~ Methods for Function diffData in Package 'cummeRbund' ~~
dim-methods ~~ Methods for Function dim in Package 'base' ~~

expressionBarplot Barplot
expressionBarplot-methods

expressionPlot Expression Plot
expressionPlot-methods

featureNames Feature names
featureNames-methods ~~ Methods for Function featureNames in Package 'cummeRbund' ~~
features Features
features-methods ~~ Methods for Function features in Package 'cummeRbund' ~~
fpkm Retrieve FPKM values
fpkm-methods ~~ Methods for Function fpkm in Package 'cummeRbund' ~~
fpkmMatrix Retrieve FPKM values as matrix
fpkmMatrix-methods ~~ Methods for Function fpkmMatrix in Package 'cummeRbund' ~~
getGene
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getGenes
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getLevels
getLevels-methods ~~ Methods for Function getLevels in Package 'cummeRbund' ~~
length-methods ~~ Methods for Function length in Package 'base' ~~
makeprobs Transform a matrix into probabilities by columns
readCufflinks readCufflinks
samples Get sample list from CuffData object
samples-methods ~~ Methods for Function samples in Package 'cummeRbund' ~~
shannon.entropy Shannon entropy

Further information is available in the following vignettes:
addFeatures

An R package for visualization and analysis of Cufflinks high-throughput sequencing data (source, pdf)

~~ An overview of how to use the package, including the most important ~~ ~~ functions ~~

Author(s)
L. Goff, C. Trapnell
Maintainer: Loyal A. Goff <lgoff@csail.mit.edu>

References
~~ Literature or other references for background information ~~

addFeatures  addFeatures

Description
Adds a data.frame of features to a the SQLite backend database.

Usage
## S4 method for signature 'CuffSet'
addFeatures(object, features, level="genes", ...)
References

None

Examples

#None yet.

count-methods  
Retrieve count values (raw and normalized)

Description

Returns a data.frame from @count slot

Details

Returns a data.frame of count values.

Value

A data.frame of count-level values for a set of features.

Methods

signature(object = "CuffData")
signature(object = "CuffFeature")
signature(object = "CuffFeatureSet")

Note

None

Author(s)

Loyal A. Goff

References

None

Examples

data(sampleData)
count(PINK1)
Description

Retrieve count values as gene by condition matrix

Usage

```r
## S4 method for signature 'CuffData'
countMatrix(object, fullnames=FALSE, sampleIdList)
## S4 method for signature 'CuffData'
repCountMatrix(object, fullnames=FALSE, repIdList)
```

Arguments

- `fullnames`: A logical value whether or not to concatenate gene_short_name and tracking_id values (easier to read labels)
- `sampleIdList`: A vector of sample names to subset the resulting matrix.
- `repIdList`: A vector of replicate names to subset the resulting replicate matrix.

Details

None.

Value

A feature x condition matrix of count values.

Note

None.

Author(s)

Loyal A. Goff

References

None.

Examples

```r
data(sampleData)
countMatrix(sampleGeneSet)
repCountMatrix(sampleGeneSet)
```
Description

Creates a ggplot2 plot object with a geom_box layer displaying summary statistics for FPKM values across samples (x).

Usage

```r
## S4 method for signature 'CuffData'
csBoxplot(object, logMode=TRUE, pseudocount=0.0001, replicates=FALSE,...)
```

Arguments

- `object`: An object of class CuffData.
- `logMode`: A logical argument to log10-transform FPKM values.
- `pseudocount`: Value added to FPKM to avoid log-transform issues.
- `replicates`: A logical value whether or not to plot individual replicates or aggregate condition values.
- `...`: Additional arguments to csBoxplot

Details

None

Value

A ggplot2 plot object with a geom_box layer.

Note

None

Author(s)

Loyal A. Goff

References

None

Examples

```r
a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Read cufflinks data and create CuffSet object
genese<-a$genes #CuffData object for all genes
csBoxplot(genes)
```
Description

Returns a ggplot2 plot object with geom_line layer plotting FPKM values over conditions faceted by k-means clustering clusters. (Euclidean). This is very crude at this point. This does not return any of the clustering information directly, but if you want it, you can retrieve it from the ggplot object returned.

Usage

## S4 method for signature 'CuffFeatureSet'
csCluster(object,k,logMode=T,method = "none",pseudocount=1,...)

Arguments

- **object**
  - An object of class CuffFeatureSet.
- **k**
  - Number of pre-defined clusters to attempt to find.
- **logMode**
  - A logical value whether or not to log-transform the FPKM values prior to clustering.
- **method**
  - Distance function to use when computing cluster solution. Default "none" will use the Jensen-Shannon distance (JSdist). Provide a function that returns a dist object on rows.
- **pseudocount**
  - Value added to FPKM to avoid log-transform issues.
- **...**
  - Additional arguments to pam.

Details

Uses 'kmeans' function.

Author(s)

Loyal A. Goff

Source

None

References

None.

Examples

data(sampleData)
csCluster(sampleGeneSet,4)
csClusterPlot

Description

Replaces the default plotting behavior of the old csCluster. Takes as an argument the output of csCluster and plots expression profiles of features facet by cluster.

Usage

csClusterPlot(clustering, pseudocount=1.0, logMode=FALSE, drawSummary=TRUE, sumFun=mean_cl_boot)

Arguments

clustering  The output of csCluster. (Must be the output of csCluster. Only this data format contains the necessary information for csClusterPlot.)
pseudocount  Value added to FPKM to avoid log transformation issues.
logMode  Logical argument whether to plot FPKM with log axis (Y-axis).
drawSummary  Logical value whether or not to draw a summary line for each cluster (by default this is the cluster mean)
sumFun  Summary function used to by drawSummary (default: mean_cl_boot)

Details

This replaces the default plotting behavior of the old csCluster() method. This was necessary so as to preserve the cluster information obtained by csCluster in a stable format. The output of csClusterPlot is a ggplot2 object of expressionProfiles faceted by cluster ID.

Value

A ggplot2 object of expressionProfiles faceted by cluster ID.

Note

None.

Author(s)

Loyal A. Goff

References

None.

Examples

data(sampleData)
myClustering<-csCluster(sampleGeneSet, k=4)
csClusterPlot(myClustering)
Description

Creates a grid graphics plot of a dendrogram of Jensen-Shannon distances between conditions of a CuffFeatureSet or CuffGeneSet object.

Usage

```r
## S4 method for signature 'CuffFeatureSet'
csdendro(object, logMode=T, pseudocount=1, replicates=FALSE)
## S4 method for signature 'CuffData'
csdendro(object, logMode=T, pseudocount=1, replicates=FALSE,...)
```

Arguments

- `object`: An object of class `CuffFeatureSet` or `CuffGeneSet`
- `logMode`: A logical argument to log10-transform FPKM values prior to plotting.
- `pseudocount`: Value to be added to FPKM for appropriate log transformation and clustering. (Avoids zero-based errors)
- `replicates`: A logical value whether or not to plot individual replicates or aggregate condition values.
- `...`: Additional arguments to csHeatmap

Details

None

Value

Returns a dendrogram object and plots that object by default.

Note

None

Author(s)

Loyal A. Goff and Cole Trapnell

References

None.

Examples

```r
data(sampleData)
csdendro(sampleGeneSet)
```
csDensity

Density plot of CuffData

Description

Creates a smoothed density plot, by sample, for log10 FPKM values from a cuffdiff run.

Usage

```r
## S4 method for signature 'CuffData'
csDensity(object, logMode=TRUE, pseudocount=0, labels, features=FALSE, replicates=FALSE, ...)
## S4 method for signature 'CuffFeatureSet'
csDensity(object, logMode=TRUE, pseudocount=0, labels, features=FALSE, replicates=FALSE, ...)
```

Arguments

- `object`: An object of class CuffData.
- `logMode`: A logical value of whether or not to log10-transform FPKM values. By default this is TRUE.
- `pseudocount`: Pseudocount value added to FPKM to avoid errors in log-transformation of true zero values.
- `labels`: A list of tracking_id values or gene_short_name values used for 'callout' points on the density plot for reference. (Not implemented yet).
- `features`: Will include all fields from 'features' slot in returned ggplot object. Useful for further manipulations of plot object using feature-level attributes (e.g. gene_type, class_code, etc)
- `replicates`: A logical value whether or not to plot individual replicates or aggregate condition values.
- `...`: Additional arguments

Details

Creates a density plot, by sample, for log10-transformed FPKM values from a cuffdiff run.

Value

A ggplot2 plot object

Note

None

Author(s)

Loyal A. Goff

References

None
Examples

```
a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Create CuffSet object from sample data
genes<-a$genes #Create CuffData object for all 'genes'
d<-csDensity(genes) #Create csDensity plot
d #Render plot
```

### csDistHeat

**Description**

Creates a ggplot plot object with a geom_tile layer of JS Distance values between samples or genes.

**Usage**

```r
## S4 method for signature 'CuffFeatureSet'
csDistHeat(object, replicates=F, samples.not.genes=T,
        logMode=T, pseudocount=1.0,
        heatscale=c(low='lightyellow',mid='orange',high='darkred'),
        heatMidpoint=NULL, ...)
```

**Arguments**

- `object` An object of class `CuffFeatureSet` or `CuffGeneSet`
- `replicates` A logical argument whether or not to use individual replicate FPKM values as opposed to condition FPKM estimates. (default: FALSE)
- `samples.not.genes` Compute distances between samples rather than genes. If False, compute distances between genes.
- `logMode` A logical argument to log10-transform FPKM values prior to plotting.
- `pseudocount` Value to be added to FPKM for appropriate log transformation and clustering. (Avoids zero-based errors)
- `heatscale` A list with min length=2, max length=3 that describe the the color scale.
- `heatMidpoint` Value for midpoint of color scale.
- `...` Additional arguments to csHeatmap

**Details**

None

**Value**

A ggplot2 plot object with a geom_tile layer to display distance between samples or genes.

**Note**

None

**Author(s)**

Loyal A. Goff, Cole Trapnell, and David Kelley
## csHeatmap

### Description

Creates a ggplot plot object with a geom_tile layer of FPKM values per feature and sample.

### Usage

```r
## S4 method for signature 'CuffFeatureSet'
csHeatmap(object, rescaling = 'none', clustering = 'none', labCol = T, labRow = T, logMode = T, pseudocount = 1.0, border = FALSE, heatscale = c(low = 'lightyellow', mid = 'orange', high = 'darkred'), heatMidpoint = NULL, fullnames = T, replicates = FALSE, method = 'none', ...)
```

```r
## S4 method for signature 'CuffGeneSet'
csFoldChangeHeatmap(object, control_condition, replicate_num = NULL, clustering = 'none', labCol = T, labRow = T, logMode = F, pseudocount = 1.0, border = FALSE, heatscale = c(low = 'steelblue', mid = 'white', high = 'tomato'), heatMidpoint = 0, fullnames = T, replicates = FALSE, method = 'none', heatRange = 3, ...)
```

### Arguments

- `object`: An object of class `CuffFeatureSet` or `CuffGeneSet`
- `control_condition`: A character argument indicating which condition should be used as the denominator for fold change. (e.g. "Day0", "Control", etc)
- `replicate_num`: If replicates == TRUE, you must specify both a control condition and a replicate number to use as the denominator.
- `rescaling`: Rescaling can either be 'row' or 'column' OR you can pass rescale a function that operates on a matrix to do your own rescaling. Default is 'none'.
- `clustering`: Clustering can either be 'row','column','none', or 'both', in which case the appropriate indices are re-ordered based on the pairwise Jensen-Shannon distance of FPKM values.
- `labCol`: A logical argument to display column labels.
- `labRow`: A logical argument to display row labels.
- `logMode`: A logical argument to log10-transform FPKM values prior to plotting.
- `pseudocount`: Value to be added to FPKM for appropriate log transformation and clustering. (Avoids zero-based errors)
- `border`: A logical argument to draw border around plot.
- `heatscale`: A list with min length=2, max length=3 that detail the low,mid,and high colors to build the color scale.
- `heatMidpoint`: Value for midpoint of color scale.
- `fullnames`: A logical value whether to use 'fullnames' (concatenated gene_short_name and gene_id) for rows in heatmap. Default [ TRUE ].
**csScatter**

replicates  A logical value whether or not to plot individual replicates or aggregate condition values.

method  Function to be used for clustering. Default is JS-distance. You can pass your own function to this argument as long as the output is an instance of the 'dist' class and is applied to the rows of the input matrix.

heatRange  Numerical argument for upper bound on log fold change to be visualized.

...  Additional arguments to csHeatmap

**Details**

None

**Value**

A ggplot2 plot object with a geom_tile layer to display FPKM values by sample (x) and feature (y)

**Note**

None

**Author(s)**

Loyal A. Goff and Cole Trapnell

**References**

None.

**Examples**

data(sampleData)
csHeatmap(sampleGeneSet)

---

**csScatter**  Scatter Plot

**Description**

A scatter plot comparing the FPKM values from two samples in a cuffdiff run.

**Usage**

```r
## S4 method for signature 'CuffData'
csScatter(object, x, y, logMode=TRUE, pseudocount=1.0, labels, smooth=FALSE, colorByStatus = FALSE, drawRug=TRUE, ...)

## S4 method for signature 'CuffData'
csScatterMatrix(object,replicates=FALSE,logMode=TRUE,pseudocount=1.0, hexbin=FALSE, useCounts=FALSE, ...)
```
Arguments

object  An object of class ('CuffData','CuffFeatureSet')
x  Sample name for x axis
y  Sample name for y axis
logMode  Logical argument to render axes on log10 scale (default: T )
replicates  Logical argument whether or not to draw individual replicate values instead of condition values. (default: T )
pseudocount  Value to add to zero FPKM values for log transformation (default: 0.0001)
smooth  Logical argument to add a smooth-fit regression line
labels  A list of tracking_ids or gene_short_names that will be 'callout' points in the plot for reference. Useful for finding genes of interest in the field. Not implemented yet.
colorByStatus  A logical argument whether or not to color the points by 'significant' Y or N. [Default = FALSE]
drawRug  A logical argument whether or not to draw the rug for x and y axes [Default = TRUE]
hexbin  Logical value whether or not to visualize overplotting with hexbin.
useCounts  Uses normalized counts instead of FPKM.
...  Additional arguments to csScatter

Details

None

Value

ggplot object with geom_point and geom_rug layers

Note

None

Author(s)

Loyal A. Goff

References

None

Examples

a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Create CuffSet object from sample data
genes<-a@genes #Create CuffData object for all genes
s<-csScatter(genes, 'hESC', 'Fibroblasts', smooth=TRUE) #Create plot object
s #render plot object
Description

Returns a matrix of 'Specificity scores' (S) defined as 1-JSD(p_g,q_i) where p_g is the Log10+1 expression profile of a gene g across all conditions j, collapsed into a probability distribution, and q_i is the unit vector of 'perfect expression' in a given condition i.

Usage

```r
## S4 method for signature 'CuffFeatureSet'
csSpecificity(object, logMode = T, pseudocount = 1, relative = FALSE, ...)
## S4 method for signature 'CuffData'
csSpecificity(object, logMode = T, pseudocount = 1, relative = FALSE, ...)
```

Arguments

- `object` An object of class CuffFeatureSet, CuffGeneSet, or CuffData.
- `logMode` A logical argument to log10-transform FPKM values prior to plotting.
- `pseudocount` Value to be added to FPKM for appropriate log transformation and clustering. (Avoids zero-based errors)
- `relative` A logical argument that when TRUE, will scale the S values from 0-1 by dividing by max(S)
- `...` Additional arguments to fpkmMatrix.

Details

None

Author(s)

Loyal A. Goff

Source

None

References

None.

Examples

data(sampleData)
csSpecificity(sampleGeneSet)
Description

Creates a volcano plot of log fold change in expression vs -log(pval) for a pair of samples (x,y)

Usage

```r
## S4 method for signature 'CuffData'
csVolcano(object, x, y, alpha=0.05, showSignificant=TRUE, features=FALSE, xlimits = c(-20, 20), ...)
## S4 method for signature 'CuffData'
csVolcanoMatrix(object, alpha=0.05, xlimits = c(-20,20), mapping=aes(),...)
```

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>object</td>
<td>An object of class CuffData, CuffFeatureSet, or CuffGeneSet</td>
</tr>
<tr>
<td>x</td>
<td>Sample name from 'samples' table for comparison</td>
</tr>
<tr>
<td>y</td>
<td>Sample name from 'samples' table for comparison</td>
</tr>
<tr>
<td>alpha</td>
<td>Provide an alpha cutoff for visualizing significant genes</td>
</tr>
<tr>
<td>showSignificant</td>
<td>A logical value whether or not to distinguish between significant features or not (by color).</td>
</tr>
<tr>
<td>features</td>
<td>Will include all fields from 'features' slot in returned ggplot object. Useful for further manipulations of plot object using feature-level attributes (e.g. gene_type, class_code, etc)</td>
</tr>
<tr>
<td>xlimits</td>
<td>Set boundaries for x limits to avoid infinity plotting errors. [Default c(-20,20)]</td>
</tr>
<tr>
<td>mapping</td>
<td>Passthrough argument for ggplot aesthetics. Can be ignored completely.</td>
</tr>
<tr>
<td>...</td>
<td>Additional arguments</td>
</tr>
</tbody>
</table>

Details

This creates a 'volcano' plot of fold change vs. significance for a pairwise comparison of genes or features across two different samples.

Value

A ggplot2 plot object

Note

None

Author(s)

Loyal A. Goff

References

None.
Examples

```r
a <- readCufflinks(system.file("extdata", package="cummeRbund")) # Create CuffSet object
genes <- a@genes # Create cuffData object for all genes
v <- csVolcano(genes,"hESC","Fibroblasts") # Volcano plot of all genes for conditions x='hESC' and y='Fibroblasts'
v # print plot
```

CuffData-class

Class "CuffData"

Description

A 'pointer' class for all information (FPKM, annotation, differential expression) for a given feature type (genes, isoforms, TSS, CDS). The methods for this function communicate directly with the SQL backend to present data to the user.

Objects from the Class

Objects can be created by calls of the form `new("CuffData", DB, tables, filters, type, idField, ...)`. 

Slots

- `DB`: Object of class "SQLiteConnection" ~~
- `tables`: Object of class "list" ~~
- `filters`: Object of class "list" ~~
- `type`: Object of class "character" ~~
- `idField`: Object of class "character" ~~

Methods

- `dim` signature(x = "CuffData"): ...
- `getFeatures` signature(object = "CuffData"): ...
- `DB` signature(object = "CuffData"): Accessor for @DB slot
- `diffTable` signature(object = "CuffData"): Create a Full table (wide format) of differential expression information for all pairwise comparisons
- `makeRnk` signature(object = "CuffData"): Internal method to create .rnk file. Should not be called directly
- `annotation` signature(object="CuffData"): Access annotation data

Note

None

Author(s)

Loyal A. Goff

References

None
CuffDist-class

See Also
None

Examples
showClass("CuffData")

CuffDist-class  Class "CuffDist"

Description
A 'pointer' class to information relative to the distribution-level tests (promoters, splicing, and relative CDS usage)

Objects from the Class
Objects can be created by calls of the form new("CuffDist", DB, table, type, idField, ...).

Slots
DB: Object of class "SQLiteConnection" ~~
table: Object of class "character" ~~
type: Object of class "character" ~~
idField: Object of class "character" ~~

Methods

   dim signature(x = "CuffDist"):
   samples signature(x = "CuffDist"):
   DB signature(object = "CuffDist"): Accessor for @DB slot

Note
None

Author(s)
Loyal A. Goff

References
None

See Also
None

Examples
showClass("CuffDist")
CuffFeature-class

Description
A 'data' container class for all FPKM, annotation, and differential expression data for a single feature (gene, isoform, TSS, or CDS).

Objects from the Class
Objects can be created by calls of the form new("CuffFeature", annotation, fpkm, diff, ...).

Slots
- annotation: Object of class "data.frame"
- fpkm: Object of class "data.frame"
- diff: Object of class "data.frame"
- repFpkm: Object of class "data.frame"
- count: Object of class "data.frame"
- genome: Object of class "character"

Methods
- fpkmMatrix signature(object="CuffFeature"): ...
- repFpkmMatrix signature(object = "CuffFeature"): ...
- length signature(x = "CuffFeature"): ...

Accessors
- annotation signature(object="CuffFeature"): Access @annotation slot
- diffData signature(object="CuffFeature"): Access @diff slot
- samples signature(object="CuffFeature"): Get vector of samples

Note
'CuffGene' is a superclass of 'CuffFeature' that links gene information for a given gene with all isoform-, TSS-, and CDS-level data for the given gene.

Author(s)
Loyal A. Goff

References
None

See Also
CuffGene
**Examples**

```r
showClass("CuffFeature")
```

---

**CuffFeatureSet-class Class "CuffFeatureSet"**

**Description**

A 'data' container class for all FPKM, annotation, and differential expression data for a set of features (genes, isoforms, TSS, CDS).

**Objects from the Class**

Objects can be created by calls of the form `new("CuffFeatureSet", annotation, fpkm, diff, ...)`.  

**Slots**

- `annotation`: Object of class "data.frame"  
- `fpkm`: Object of class "data.frame"  
- `diff`: Object of class "data.frame"  
- `repFpkm`: Object of class "data.frame"  
- `count`: Object of class "data.frame"  
- `genome`: Object of class "character"  

**Methods**

- `diffData` signature(`object = "CuffFeatureSet"`): ...
- `featureNames` signature(`object = "CuffFeatureSet"`): ...
- `features` signature(`object = "CuffFeatureSet"`): ...
- `fpkmMatrix` signature(`object = "CuffFeatureSet"`): ...
- `repFpkmMatrix` signature(`object = "CuffFeatureSet"`): ...
- `countMatrix` signature(`object = "CuffFeatureSet"`): ...
- `samples` signature(`object = "CuffFeatureSet"`): ...
- `length` signature(`object = "CuffFeatureSet"`): ...

**Accessors**

- `annotation` signature(`object="CuffFeatureSet"`): Access @annotation slot

**Note**

None.

**Author(s)**

Loyal A. Goff
**CuffGene-class**

**References**

None.

**See Also**

*CuffGeneSet*

**Examples**

```r
showClass("CuffFeatureSet")
```

---

**Description**

A 'data' container class for all FPKM, annotation, and differential expression Data (as well as for all linked features) for a given gene.

**Objects from the Class**

Objects can be created by calls of the form `new("CuffGene", id, isoforms, TSS, CDS, promoters, splicing, relCDS, annotation, fpkm, diff, ...)`.

**Slots**

- `id`: Object of class "character"~~
- `isoforms`: Object of class "CuffFeature"~~
- `TSS`: Object of class "CuffFeature"~~
- `CDS`: Object of class "CuffFeature"~~
- `promoters`: Object of class "CuffFeature"~~
- `relCDS`: Object of class "CuffFeature"~~
- `splicing`: Object of class "CuffFeature"~~
- `annotation`: Object of class "data.frame"~~
- `genome`: Object of class "character"~~
- `fpkm`: Object of class "data.frame"~~
- `diff`: Object of class "data.frame"~~
- `features`: Object of class "data.frame"~~

**Extends**

Class "CuffFeature", directly.

**Methods**

- `length` signature(object="CuffFeature"): Part of length validation (internal use only)
- `makeGeneRegionTrack` signature(object="CuffFeature"): Creates a GeneRegionTrack object (see package Gviz) from a CuffGene object.
- `genePlot` signature(object="CuffFeature"): Internal use only.
- `csPie` signature(object="CuffGene"): Allows for visualization of relative isoform proportion as a pie chart by condition (or optionally as stacked bar charts by adding `+ coord_cartesian()`
Accessors

genes signature(object = "CuffGene"): Access @genes slot

isoforms signature(object = "CuffGene"): Access @isoforms slot

TSS signature(object = "CuffGene"): Access @TSS slot

CDS signature(object = "CuffGene"): Access @CDS slot

promoters signature(object = "CuffGene"): Access @CDS slot

relCDS signature(object = "CuffGene"): Access @CDS slot

splicing signature(object = "CuffGene"): Access @CDS slot

features signature(object = "CuffGene"): Access @features slot

Note

None.

Author(s)

Loyal A. Goff

References

None.

See Also

CuffFeature

Examples

showClass("CuffGene")

--

CuffGeneSet-class  Class "CuffGeneSet"

Description

A 'data' container class for all FPKM, annotation, and differential expression data (an associated features) for a given set of genes.

Objects from the Class

Objects can be created by calls of the form new("CuffGeneSet", annotation, fpkm, diff, ...).
Slots

ids: Object of class "character"
isoforms: Object of class "CuffFeatureSet"
TSS: Object of class "CuffFeatureSet"
CDS: Object of class "CuffFeatureSet"
promoters: Object of class "CuffFeatureSet"
relCDS: Object of class "CuffFeatureSet"
splicing: Object of class "CuffFeatureSet"
annotation: Object of class "data.frame"
fpkm: Object of class "data.frame"
diff: Object of class "data.frame"

Extends

Class "CuffFeatureSet", directly.

Methods

No methods defined with class "CuffGeneSet" in the signature.

Accessors

genes signature(object = "CuffGeneSet"): Access @genes slot
isoforms signature(object = "CuffGeneSet"): Access @isoforms slot
TSS signature(object = "CuffGeneSet"): Access @TSS slot
CDS signature(object = "CuffGeneSet"): Access @CDS slot
promoters signature(object = "CuffGeneSet"): Access @promoters slot
relCDS signature(object = "CuffGeneSet"): Access @relCDS slot
splicing signature(object = "CuffGeneSet"): Access @splicing slot

Note

None.

Author(s)

Loyal A. Goff

References

None.

See Also

CuffFeatureSet

Examples

showClass("CuffGeneSet")
Class "CuffSet"

Description
A 'pointer' class to connect to, and retrieve data from the SQLite backend database.

Objects from the Class
Objects can be created by calls of the form new("CuffSet", DB, conditions, genes, isoforms, TSS, CDS, promoters, splicing, relCDS, ...). Available methods are primary accessors to retrieve CuffGeneSet or CuffGene objects for manipulation.

Slots
- **DB**: Object of class "SQLiteConnection"
- **conditions**: Object of class "data.frame"
- **genes**: Object of class "CuffData"
- **isoforms**: Object of class "CuffData"
- **phenoData**: Object of class "data.frame"
- **TSS**: Object of class "CuffData"
- **CDS**: Object of class "CuffData"
- **promoters**: Object of class "CuffDist"
- **splicing**: Object of class "CuffDist"
- **relCDS**: Object of class "CuffDist"
- **conditions**: Object of class "data.frame"

Methods
- [ signature(x = "CuffSet")]: ...

Accessors
- **DB** signature(object="CuffSet"): Access @DB slot
- **genes** signature(object = "CuffSet"): Access @genes slot
- **isoforms** signature(object = "CuffSet"): Access @isoforms slot
- **TSS** signature(object = "CuffSet"): Access @TSS slot
- **CDS** signature(object = "CuffSet"): Access @CDS slot
- **promoters** signature(object = "CuffSet"): Access @promoters slot
- **splicing** signature(object = "CuffSet"): Access @splicing slot
- **relCDS** signature(object = "CuffSet"): Access @relCDS slot
- **varModel** signature(object = "CuffSet"): Access varModel info

Note
None.
**diffData**

**Author(s)**
Loyal A. Goff

**References**
None.

**See Also**
None.

**Examples**
```r
showClass("CuffSet")
```

---

**diffData**  
*Differential comparison data*

**Description**
An accessor method to retrieve differential expression data from a `CuffData`, `CuffFeatureSet`, or `CuffFeature` object.

**Usage**
```r
## S4 method for signature 'CuffData'
diffData(object, x, y, features=FALSE)
## S4 method for signature 'CuffData'
diffTable(object, logCutoffValue=99999)
```

**Arguments**

- `object`  
  An object of class (`CuffData` or `CuffFeatureSet`)

- `x`  
  Optional, if `x` and `y` are both missing, data for all pairwise differential comparisons are returned, otherwise if `x` and `y` are sample names from the `samples` table, than only differential data pertaining to those two samples are returned.

- `y`  
  See `x`

- `features`  
  A logical value that returns all feature-level data as part of data.frame when true. object must be of class `CuffData`.

- `logCutoffValue`  
  Cutoff value for FC estimates to convert to [-]Inf values. Should never really be needed...

- `...`  
  Additional arguments.

**Details**
None

**Value**
A data.frame object
Dimensionality Reduction

Note
None

Author(s)
Loyal A. Goff

References
None

Examples

```r
data(sampleData)
diff <- diffData(sampleGeneSet) #returns a dataframe of differential expression data from sample CuffGeneSet object.
```

---

Dimensionality Reduction

*Dimensionality reduction utilities*

Description
Dimensionality reduction plots for feature selection and extraction for cummeRbund

Usage

```r
## S4 method for signature 'CuffData'
MDSplot(object, replicates = FALSE, logMode = TRUE, pseudocount = 1.0)
## S4 method for signature 'CuffData'
PCAplot(object, x = "PC1", y = "PC2", replicates = FALSE, pseudocount = 1.0, scale = TRUE, showPoints = TRUE, ...)
```

Arguments

- `object`: The output of class CuffData from which to draw expression estimates. (e.g. genes(cuff))
- `x`: For PCAplot, indicates which principal component is to be presented on the x-axis (e.g. "PC1", "PC2", "PC3", etc)
- `y`: See `x`.
- `pseudocount`: Value added to FPKM to avoid log transformation issues.
- `logMode`: Logical value whether or not to use log-transformed expression estimates (default: TRUE)
- `replicates`: A logical value to indicate whether or not individual replicate expression estimates will be used.
- `scale`: For PCAplot, a logical value passed directly to prcomp.
- `showPoints`: For PCAplot, a logical value whether or not to display individual gene values on final PCA plot.
- `...`: Additional passthrough arguments (may not be fully implemented yet).
**dispersionPlot**

**Details**

These methods attempt to project a matrix of expression estimates across conditions and/or replicates onto a smaller number of dimensions for feature selection, feature extraction, and can also be useful for outlier detection.

**Value**

A ggplot2 object.

**Note**

None.

**Author(s)**

Loyal A. Goff

**References**

None.

**Examples**

cuff <- readCufflinks(system.file("extdata", package="cummerbund")) # Create CuffSet object from sample data
p <- PCAplot(genes(cuff), x="PC2", y="PC3", replicates=TRUE)
m <- MDSplot(genes(cuff), replicates=TRUE)
p # Render PCA plot
m # Render MDS plot

---

**dispersionPlot**

*Mean count vs dispersion plot*

**Description**

A scatter plot comparing the mean counts against the estimated dispersion for a given level of features from a cuffdiff run.

**Usage**

```r
## S4 method for signature 'CuffData'
dispersionPlot(object)
## S4 method for signature 'CuffSet'
dispersionPlot(object)
```

**Arguments**

- `object` An object of class ('CuffData')

**Details**

None
Value

ggplot object with geom_point layer

Note

None

Author(s)

Loyal A. Goff

References

None

Examples

```r
a <- readCufflinks(system.file("extdata", package="cummeRbund"))  # Create CuffSet object from sample data
genes <- genes(a)  # Create CuffData object for all genes
d <- dispersionPlot(genes)  # Create plot object
d  # render plot object
```

Description

Returns a data.frame of distribution-level test values from a CuffDist object (@promoters, @splicing, @relCDS)

Usage

```r
## S4 method for signature 'CuffDist'
distValues(object)
```

Arguments

- `object`: An object of class `CuffDist`
- `...`: Additional arguments to `distValues`

Details

None

Value

Returns a data.frame of distribution-level test values.

Note

None
Exploratory Analysis

Author(s)

Loyal A. Goff

References

None

See Also

None

Examples

a <- readCufflinks(system.file("extdata", package="cummeRbund")) # Read cufflinks data and create CuffSet object
distValues(a@promoters) # returns data.frame of values from CuffDist object in slot 'promoters'

---

Exploratory Analysis  Methods for Exploratory Analysis in cummeRbund

Description

Exploratory analysis methods for cummeRbund RNA-Seq data.

Usage

## S4 method for signature 'CuffData'
csnMF(object, k, logMode = T, pseudocount = 1, maxiter = 1000, replicates = FALSE, fullnames = FALSE)
## S4 method for signature 'CuffFeatureSet'
csnMF(object, k, logMode = T, pseudocount = 1, maxiter = 1000, replicates = FALSE, fullnames = FALSE)

Arguments

object The output of class CuffData or CuffFeatureSet from which to draw expression estimates. (e.g. genes(cuff) or custom feature set via getGenes() or getFeatures())
k rank value for factorization
logMode Logical value whether or not to use log-transformed FPKM values. [Default: TRUE]
pseudocount Value added to FPKM to avoid log transformation issues.
maxiter Maximum number of iterations for factorization [Default: 1000]
replicates A logical value to indicate whether or not individual replicate expression estimates will be used.
fullnames Logical passthrough value to fpkmMatrix whether or not to concatenate gene_short_name with tracking_id. [Default: FALSE]

Details

csnMF is a convenience method to invoke the nmf() method from package:NMFN. This performs non-negative matrix factorization on the provided data and can be useful for many downstream applications.
expressionBarplot

Value

csNMF returns W, H - decomposed matrices of input FPKM values. (See package:NMFN for details)

Note

None.

Author(s)

Loyal A. Goff

References

None.

Examples

data(sampleData)
csNMF(sampleGeneSet,4)

expressionBarplot   Barplot

Description

A barplot of FPKM values with confidence intervals for a given gene, set of genes, or features of a gene (e.g. isoforms, TSS, CDS, etc).

Usage

## S4 method for signature 'CuffFeatureSet'
expressionBarplot(object, logMode=TRUE, pseudocount=1.0, showErrorbars=TRUE, showStatus=TRUE, replicates=FALSE, ...)

Arguments

object            An object of class ('CuffFeatureSet', 'CuffGeneSet', 'CuffFeature', 'CuffGene')
logMode           A logical value whether or not to draw y-axis on log10 scale. Default = FALSE.
pseudocount       Numerical value added to each FPKM during log-transformation to avoid errors.
showErrorbars     A logical value whether or not to draw error bars. Default = TRUE
showStatus        A logical value whether or not to draw visual queues for quantification status of a given gene:condition. Default = TRUE
replicates        A logical value whether or not to plot individual replicates or aggregate condition values.
...                Additional arguments.

Details

None
**expressionPlot**

**Value**

A ggplot2 plot object

**Note**

Need to implement logMode and features for this plotting method.

**Author(s)**

Loyal A. Goff

**References**

None

**Examples**

```r
data(sampleData)
PINK1 # sample CuffFeature object
expressionBarplot(PINK1) # Barplot of PINK1 FPKM values
expressionBarplot(PINK1@isoforms) # Barplot of PINK1 FPKM values faceted by isoforms
```

---

**Description**

A line plot (optionally with confidence intervals) detailing FPKM expression levels across conditions for a given gene(s) or feature(s)

**Usage**

```r
## S4 method for signature 'CuffFeature'
expressionPlot(object, logMode=FALSE, pseudocount=1.0, drawSummary=FALSE, sumFun=mean_cl_boot, showErrorbars=TRUE, showStatus=TRUE, replicates=FALSE, facet=TRUE, ...)
```

**Arguments**

- `object`: An object of class (`'CuffFeature'` or `'CuffGene'`)
- `logMode`: A logical value to draw y-axis (FPKM) on log-10 scale. Default = FALSE.
- `pseudocount`: A numeric value added to FPKM to avoid errors on log-10 transformation.
- `drawSummary`: A logical value. Draws a 'summary' line with mean FPKM values for each condition.
- `sumFun`: Function used to determine values for summary line. Default = mean_cl_boot
- `showErrorbars`: A logical value whether or not to draw error bars.
- `showStatus`: A logical value whether or not to draw visual queues for quantification status of a given gene:condition. Default = TRUE
- `replicates`: A logical value whether or not to plot individual replicates or aggregate condition values.
- `facet`: A logical value whether or not to facet the plot by feature id (default=TRUE).
- `...`: Additional arguments
featureNames

Details
None

Value
A ggplot2 plot object

Note
None

Author(s)
Loyal A. Goff

References
None

Examples

data(sampleData)
PINK1 # sample CuffFeature object
expressionPlot(PINK1) # Line plot of PINK1 FPKM values
expressionPlot(PINK1@isoforms) # Line plot of PINK1 FPKM values faceted by isoforms

---

featureNames  Feature names

Description
Retrive a vector of feature names from a 'CuffData' or 'CuffFeatureSet' object

Usage

## S4 method for signature 'CuffData'
featureNames(object)

Arguments

object An object of class ('CuffData' or 'CuffFeatureSet')

Details
None

Value
A list of feature names

Note
None
Author(s)
Loyal A. Goff

References
None

Examples

data(sampleData)
featureNames(sampleGeneSet)

<table>
<thead>
<tr>
<th>features</th>
<th>Features</th>
</tr>
</thead>
</table>

Description
Returns a data frame of features from a CuffGene object

Usage
```r
## S4 method for signature 'CuffGene'
features(object)
```

Arguments

| object | An object of class ('CuffGene') |

Details
None

Value
A data.frame of feature-level information

Note
None

Author(s)
Loyal A. Goff

References
None

Examples

data(sampleData)
features(PINK1)
Description

A helper function to retrieve the gene_ids given a 'lookup' value (e.g. gene_short_name, isoform_id, etc). Utility to search for gene_id and gene_short_name given a single 'query' string (e.g. query='pink1' will return all genes with 'pink1' (case-insensitive) in the gene_short_name field.

Usage

```r
## S4 method for signature 'CuffSet'
findGene(object, query)
```

Arguments

- `object` An object of class 'CuffSet' (Primary 'pointer' object for Cufflinks data).
- `query` A character string for which you would like to retrieve corresponding gene_id values.

Details

None.

Value

Returns a data.frame of gene_id and gene_short_name values corresponding to genes from which 'query' matches

Note

Right now, this does not return an error if it cannot find a gene. (this is probably a bad thing...)

Author(s)

Loyal A. Goff

References

None.

Examples

```r
cuff<-readCufflinks(system.file("extdata", package="cummeRbund")) #Read cufflinks data and create master Cuff
myQuery<-'pink1'
findGene(cuff,myQuery) # Retrieve gene_id values for any genes matching 'pink1'
```
**Description**

Returns a CuffGeneSet containing n genes with the most similar expression profiles to gene/profile x.

**Usage**

```r
## S4 method for signature 'CuffSet'
findSimilar(object, x, n, distThresh, returnGeneSet=TRUE, ...)
```

**Arguments**

- **object**
  A object of class 'CuffSet'

- **x**
  A 'gene_id' or 'gene_short_name' from which to look up an expression profile OR a vector of expression values to compare all genes (vector must have same length and order of 'samples')

- **n**
  Number of similar genes to return

- **distThresh**
  A thresholding value on which to filter results based on JS-distance (e.g. A distThresh of 1.0 will return all genes, 0.0 will return those genes with 'perfect identity' to the gene of interest.)

- **returnGeneSet**
  A logical value whether to return a CuffGeneSet object [default] or a distance-ranked data frame of similar genes. The latter is useful if you want to explore the returned list based on distances.

- **...**
  Additional arguments to fpkmMatrix call within findSimilar (e.g. fullnames=T)

**Details**

By default, returns a CuffGeneSet object with n similar genes. This may change in the future.

**Value**

A CuffGeneSet object of n most similar genes to x.

**Note**

None

**Author(s)**

Loyal A. Goff

**References**

None

**Examples**

```r
a<-readCufflinks(system.file("extdata", package="cummeRbund"))
mySimilarGenes<-findSimilar(a,"PINK1",10)
```
fpkmMatrix

fpkm-methods

Retrieve FPKM values

Description
Returns a data.frame from @FPKM slot

Details
Returns a data.frame of FPKM values.

Value
A data.frame of FPKM-level values for a set of features.

Methods
signature(object = "CuffData")
signature(object = "CuffFeature")
signature(object = "CuffFeatureSet")

Note
None

Author(s)
Loyal A. Goff

References
None

Examples

data(sampleData)
fpkm(PINK1)

fpkmMatrix

Description
Retrieve FPKM values as gene by condition (fpkmMatrix) or gene by replicate (repFpkmMatrix) matrix
getFeatures

Usage

## S4 method for signature 'CuffData'
fpkmMatrix(object,fullnames=FALSE,sampleIdList)
## S4 method for signature 'CuffData'
repFpkmMatrix(object,fullnames=FALSE,repIdList)

Arguments

object          An object of class ('CuffData', 'CuffFeatureSet', 'CuffGeneSet', 'CuffGene', or 'CuffFeature')
fullnames       A logical value whether or not to concatenate gene_short_name and tracking_id values (easier to read labels)
sampleIdList    A vector of sample names to subset the resulting matrix.
repIdList       A vector of sample names to subset the resulting matrix.

Details

None.

Value

A feature x condition matrix of FPKM values.

Note

None

Author(s)

Loyal A. Goff

References

None.

Examples

data(sampleData)
fpkmMatrix(sampleGeneSet)
repFpkmMatrix(sampleGeneSet)

getFeatures

Description

Primary accessor from a CuffSet object to retrieve all related information for >1 (MANY) given FEATURES, indexed by tracking id.
getGene

Usage

## S4 method for signature 'CuffSet'
getFeatures(object, featureIdList, sampleIdList=NULL, level='isoforms')

Arguments

object An object of class 'CuffSet' (Primary 'pointer' object for Cufflinks data).
featureIdList A vector of 'isoform_id', 'TSS_group_id', or 'CDS_id' to identify which features for which you would like to retrieve all information.
sampleIdList A vector of sample names used to subset or re-order samples in returned object
level Feature level to be queried for significance (must be one of c('isoforms','TSS','CDS'))

Details

None.

Value

Returns a CuffFeatureSet object containing all related information for a given set of tracking_id values

Note

Right now, this does not return an error if it cannot find a gene. (this is probably a bad thing...)

Author(s)

Loyal A. Goff

References

None.

Examples

cuff<-readCufflinks(system.file("extdata", package="cummeRbund")) #Read cufflinks data and create master Cuff Links
sample.isoform.ids<-sample(featureNames(isoforms(cuff)),10)
myGene<-getFeatures(cuff,sample.isoform.ids) # Retrieve all information for a set of 10 sampled features.

getGene

getGene

Description

Primary accessor from a CuffSet object to retrieve all related information for 1 (one) given gene, indexed by gene_id or gene_short_name.

Usage

## S4 method for signature 'CuffSet'
getGene(object, geneId, sampleIdList=NULL)
getGeneId

Arguments

object    An object of class 'CuffSet' (Primary 'pointer' object for Cufflinks data).
geneId    A character string to identify which gene for which you would like to retrieve all information.
sampleIdList A vector of sample names used to subset or re-order samples in returned object

Details

None.

Value

Returns a CuffGene object containing all related information for a given gene_id or gene_short_name

Note

Right now, this does not return an error if it cannot find a gene. (this is probably a bad thing...)

Author(s)

Loyal A. Goff

References

None.

Examples

a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Read cufflinks data and create master CuffSet
myGene<-getGene(a,"PINK1") # Retrieve all information for gene "PINK1"

getGeneId  getGene

Description

A helper function to retrieve the gene_ids for a given list of feature ids (e.g. isoform_ids, tss_group_ids, or CDS_ids). This should not be called directly by the user

Usage

## S4 method for signature 'CuffSet'
getGeneId(object, idList)

Arguments

object    An object of class 'CuffSet' (Primary 'pointer' object for Cufflinks data).
idList    A character string to identify the identifiers for which you would like to retrieve corresponding gene_id values.
getGenes

Details
None.

Value
Returns a vector of gene_id values corresponding to genes from which idList are sub-features.

Note
Right now, this does not return an error if it cannot find a gene. (this is probably a bad thing...)

Author(s)
Loyal A. Goff

References
None.

Examples
cuff<-readCufflinks(system.file("extdata", package="cumRbund")) #Read cufflinks data and create master Cuff sampleFeatureIds<-sample(featureNames(isoforms(cuff)),10) correspondingGeneIds<-getGeneId(cuff,sampleFeatureIds) # Retrieve gene_id values for parent genes of sampleFeatureIds.

description

Primary accessor from a CuffSet object to retrieve all related information for >1 (MANY) given genes, indexed by gene_id or gene_short_name.

Usage
## S4 method for signature 'CuffSet'
getGenes(object, geneIdList, sampleIdList=NULL)

Arguments

- **object**: An object of class 'CuffSet' (Primary 'pointer' object for Cufflinks data).
- **geneIdList**: A vector of gene_ids or gene_short_name to identify which genes for which you would like to retrieve all information.
- **sampleIdList**: A vector of sample names used to subset or re-order samples in returned object

Details
None.

Value
Returns a CuffGeneSet object containing all related information for a given set of gene_id or gene_short_name values.
getLevels

Note
Right now, this does not return an error if it cannot find a gene. (this is probably a bad thing...)

Author(s)
Loyal A. Goff

References
None.

Examples
a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Read cufflinks data and create master CuffSets
data(sampleData)
sampleIDs
myGene<-getGenes(a,sampleIDs) # Retrieve all information for a set of 20 'sample' genes.

getLevels

Description
Returns a list of samples as levels. This should not be called directly by user.

Usage
## S4 method for signature 'CuffData'
getLevels(object)

Arguments
object An object of class 'CuffData' or 'CuffFeatureSet' or 'CuffFeature'

Details
For internal usage only.

Value
A vector of sample names as factors.

Note
None.

Author(s)
Loyal A. Goff

References
None.
getRepLevels

Description
Returns a list of replicate samples as levels. This should not be called directly by user.

Usage
## S4 method for signature 'CuffData'
getRepLevels(object)

Arguments
object
An object of class 'CuffSet' or 'CuffData'

Details
For internal usage only.

Value
A vector of replicate names as factors.

Note
None.

Author(s)
Loyal A. Goff

References
None.

getSig

Description
Returns the identifiers of significant genes in a vector format.

Usage
## S4 method for signature 'CuffSet'
getSig(object,x,y,alpha=0.05,level='genes',method="BH", useCuffMTC=FALSE)
**getSig**

**Arguments**

- **object**: A CuffSet object (e.g. cuff)
- **x**: Optional argument to restrict significance results to one pairwise comparison. Must be used with a 'y' argument to specify the other half of the pair.
- **y**: See x.
- **alpha**: An alpha value by which to filter multiple-testing corrected q-values to determine significance
- **level**: Feature level to be queried for significance (must be one of c('genes','isoforms','TSS','CDS'))
- **method**: Multiple testing method to be used for correction. (default: "BH")
- **useCuffMTC**: Logical vector whether or not to use the multiple-testing corrected q-values from the cuffdiff analysis directly, or calculate new q-values from a subset of tests.

**Details**

This is a convenience function to quickly retrieve vectors of identifiers for genes or features that were determined to be significantly regulated between conditions by cuffdiff. This function only returns tracking IDs that correspond to tests with an 'OK' status from cuffdiff, NOTEST values are ignored. By default getSig returns a vector of IDs for all pairwise comparisons together. If you specify an 'x' AND 'y' values as sample names, then only the features that are significant in that particular pairwise comparison are reported, after appropriate multiple testing correction of output p-values.

**Value**

A vector of feature IDs.

**Note**

None.

**Author(s)**

Loyal A. Goff

**References**

None.

**Examples**

```r
a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Read cufflinks data in sample directory and creates CuffSet object
mySig<-getSig(a,x='hESC',y='Fibroblasts',alpha=0.05,level='genes')
head(mySig)
```
getSigTable

Description
Returns the identifiers of significant genes in a test table - like format.

Usage
```r
## S4 method for signature 'CuffSet'
getSigTable(object, alpha=0.05, level='genes')
```

Arguments
- **object**: A CuffSet object (e.g. cuff)
- **alpha**: An alpha value by which to filter multiple-testing corrected q-values to determine significance
- **level**: Feature level to be queried for significance (must be one of c('genes','isoforms','TSS','CDS'))

Details
This is a convenience function to quickly retrieve lists of identifiers for genes or features that were determined to be significantly regulated between conditions by cuffdiff. This function only returns tracking IDs that correspond to tests with an 'OK' status from cuffdiff, NOTEST values are ignored or reported as NA. By default getSig returns a table of genes x conditions, where the column names represent the pairwise comparisons from the cuffdiff analysis. The values in the table are 1 for features that are significant for this comparison and 0 for genes that are not, any failed tests are reported as <NA>. Only includes the features that are significant in at least one comparison.

Value
A data.frame of pairwise test results.

Note
None.

Author(s)
Loyal A. Goff

References
None.

Examples
```r
a<-readCufflinks(system.file("extdata", package="cummeRbund")) # Read cufflinks data in sample directory and creates CuffSet object

mySigTable<-getSigTable(a, alpha=0.05, level='genes')
head(mySigTable)
```
JSDist  

Jensen-Shannon distance on columns

Description

JSDist takes a matrix of expression probabilities (calculated directly or output from makeprobs()) and returns a dist object of the pairwise Jensen-Shannon distances between columns.

Usage

JSDist(mat,...)

Arguments

mat A matrix of expression probabilities (e.g. from makeprobs())
...

Details

Returns pairwise Jensen-Shannon distance (in the form of a dist object) for a matrix of probabilities (by column).

Value

A dist object of pairwise J-S distances between columns.

Note

None

Author(s)

Loyal A. Goff

References

None

Examples

mat<-matrix(sample(1:50,50),10)  
probs<-makeprobs(mat)  
js.distance<-JSDist(probs)
**JSdistFromP**  
Jensen-Shannon distance on rows from a pre-defined vector of probabilities

**Description**

JSdist takes a matrix of expression probabilities (calculated directly or output from makeprobs()) and returns a matrix of Jensen-Shannon distances between individual rows and a specific vector of probabilities (q).

**Usage**

`JSdistFromP(mat,q)`

**Arguments**

- `mat`: A matrix of expression probabilities (e.g. from makeprobs())
- `q`: A vector of expression probabilities.

**Details**

Returns Jensen-Shannon distance for each row of a matrix of probabilities against a provided probability distribution (q).

**Value**

A vector of JS distances

**Note**

None

**Author(s)**

Loyal A. Goff

**References**

None

**Examples**

```r
mat<-matrix(sample(1:50,50),10)
q<-c(100,4,72,8,19)
q<-q/sum(q)
js.distance<-JSdistFromP(mat,q)
```
Description

Returns the Jensen-Shannon Distance (square root of JS divergence) between two probability vectors.

Usage

\[ \text{JSdistVec}(p, q) \]

Arguments

- `p`: A vector of probabilities
- `q`: A vector of probabilities

Details

Should not be called directly by user.

Value

Returns the JS distance as a numeric

Note

None

Author(s)

Loyal A. Goff

References

None

Examples

\[
\begin{align*}
p & \leftarrow \text{sample}(1:5000, 20) \\
q & \leftarrow \text{sample}(1:5000, 20) \\
p & \leftarrow \text{makeprobsvec}(p) \\
q & \leftarrow \text{makeprobsvec}(q) \\
\text{JSdistVec}(p, q)
\end{align*}
\]
Description

This function takes a matrix of expression values (must be greater than 0) and returns a matrix of probabilities by column. This is a required transformation for the Jensen-Shannon distance which is a metric that operates on probabilities.

Usage

makeprobs(a)

Arguments

a

A matrix of expression values (values must be greater than 0).

Details

To make a matrix of probabilities by row, use t() to transpose prior to calling makeprobs.

Value

A matrix of expression probabilities by column.

Note

None

Author(s)

Loyal A. Goff

References

None

Examples

myMat<-matrix(sample(1:50,50),10)
probs<-makeprobs(myMat)
**Description**

Sums a vector of numerics and divides by the sum

**Usage**

`makeprobsvec(p)`

**Arguments**

*p*  
A vector of numerics

**Details**

None

**Value**

A vector of probabilities

**Note**

Should not be called directly by user.

**Author(s)**

Loyal A. Goff

**References**

None

**Examples**

```r
p <- sample(1:5000, 20)  
makeprobsvec(p)
```
MAplot

Description

Creates an M vs A plot (Avg intensity vs log ratio) for a given pair of conditions across all fpkms.

Usage

```r
## S4 method for signature 'CuffData'
MAplot(object, x, y, logMode=TRUE, pseudocount=1, smooth=FALSE, useCount=FALSE)
```

Arguments

- `object`: An object of class 'CuffData'.
- `x`: Sample name from 'samples' table for comparison.
- `y`: Sample name from 'samples' table for comparison.
- `logMode`: A logical argument to log10-transform FPKM values prior to plotting.
- `pseudocount`: Value to be added to FPKM for appropriate log transformation and clustering. (Avoids zero-based errors)
- `smooth`: Logical argument whether or not to draw a smoothed line fit through data.
- `useCount`: Logical argument whether or not to use mean counts instead of FPKM values.

Details

None.

Value

Returns a ggplot MvsA plot object.

Note

None.

Author(s)

Loyal A. Goff and Cole Trapnell.

References

None.

Examples

```r
a<readCufflinks(system.file("extdata", package="cummeRbund")) #Create CuffSet object from sample data
genesc<--a@genes #Create CuffData object for all 'genes'
d<--MAplot(genes,'hESC','Fibroblasts') #Create csDensity plot
d #Render plot
```
**PINK1**

**Description**

A sample 'CuffGene' dataset

**Usage**

data(sampleData)

**Format**

PINK1 is a CuffGene object (extends CuffFeature) with all sample gene-, isoform-, TSS-, and CDS-level data for the gene 'PINK1'.

**Details**

Sample CuffGene data for gene 'PINK1'

**Source**

None

**References**

None

**Examples**

data(sampleData)
PINK1

---

**QCplots**

**Quality Control visualizations**

**Description**

A collection of ggplot2 visualizations for quality control assessment of cuffdiff output.

- fpkmSCVPlot: A measure of cross-replicate variability, the squared coefficient of variation is a normalized measure of variance between empirical replicate FPKM values per condition, across the range of FPKM estimates.

**Usage**

```r
## S4 method for signature 'CuffData'
fpkmSCVPlot(object, FPKMLowerBound = 1, showPool = FALSE)
```
**Arguments**

- **object**
  An object of class CuffData.
- **FPKMLowerBound**
  A lower limit cutoff for FPKM values from which a fit of squared Coefficient of variation (default: 1)
- **showPool**
  Logical argument whether to display variability across all replicates independent of condition (TRUE) or the cross-replicate variability for each condition (FALSE)

**Details**

None

**Value**

A ggplot2 plot object with a geom_box layer.

**Note**

None

**Author(s)**

Loyal A. Goff

**References**

None

**Examples**

```r
a <- readCufflinks(system.file("extdata", package="cummeRbund")) # Read cufflinks data and create CuffSet object
genes <- a@genes # CuffData object for all genes
csBoxplot(genes)
```

---

**Description**

This initializes the backend SQLite table and provides a DB connection for all downstream data analysis.

**Usage**

```r
readCufflinks(dir = getwd(), dbfile = "cuffData.db", gtfFile = NULL,
runInfoFile = "run.info", repTableFile = "read_groups.info",
geneFPKM = "genes.fpkm_tracking", geneDiff = "gene.exp.diff", geneCount="genes.count_tracking",
geneRep="genes.read_group_tracking",
isoformFPKM = "isoforms.fpkm_tracking", isoformDiff = "isoform.exp.diff", isoformCount="isoforms.count_tracking",
isoformRep="isoforms.read_group_tracking",
TSSFPKM = "tss_groups.fpkm_tracking", TSSDiff = "tss_group.exp.diff", TSSCount="tss_groups.count_tracking",
TSSRep="tss_groups.read_group_tracking",
CDSFPKM = "cds.fpkm_tracking", CDSExpDiff = "cds_exp.diff", CDSCount="cds.count_tracking", CDSRep="cds.read_group_tracking",
CDSDiff = "cds.diff",
```
promoterFile = "promoters.diff",
splicingFile = "splicing.diff",
varModelFile = "var_model.info",
driver = "SQLite",
genome = NULL,
rebuild = FALSE,verbose=FALSE, ...)

Arguments

dir Directory in which all CuffDiff output files can be located. Defaults to current working directory.
dbFile Name of backend database. Default is 'cuffData.db'
gtfFile Path to .gtf file used in cuffdiff analysis. This file will be parsed to retrieve transcript model information.
runInfoFile run.info file
repTableFile read_groups.info file
geneFPKM genes.fpkm_tracking file
geneDiff gene_exp.diff file
geneCount genes.count_tracking file
geneRep genes.read_group_tracking file
isoformFPKM isoforms.fpkm_tracking file
isoformDiff isoform_exp.diff file
isoformCount isoforms.count_tracking file
isoformRep isoform.read_group_tracking file
TSSFPKM tss_groups.fpkm_tracking file
TSSDiff tss_group_exp.diff file
TSSCount tss_groups.count_tracking file
TSSRep tss_groups.read_group_tracking file
CDSFPKM cds.fpkm_tracking file
CDSExpDiff cds_exp.diff file
CDSCount cds.count_tracking file
CDSRep cds.read_group_tracking file
CDSDiff cds.diff file (distribution tests on CDS)
promoterFile promoters.diff file (distribution tests on promoters)
splicingFile splicing.diff (distribution tests on isoforms)
varModelFile varModel.info (emitted in cuffdiff >= v2.1)
driver Driver for backend database. (Currently only "SQLite" is supported).
genome A character string indicating to which genome build the .gtf annotations belong (e.g. 'hg19' or 'mm9')
rebuild A logical argument to rebuild database backend.
verbose A logical argument for super verbose reporting (As if it wasn’t enough already!)
... Additional arguments to readCufflinks
repFpkm-methods

Details

This is the initialization function for the cummeRbund package. It creates the SQLite backend database, populates the data, and provides a connection object for all future interactions with the dataset. Once the initial build is complete, this function will default to using the database for all future sessions. IMPORTANT: - Each R session should begin with a call to readCufflinks to re-open the connection to the database. - Should any connectivity issues to the database arise, another call to readCufflinks should create a new connection object and repair any issue. - The database can always be rebuild (using rebuild=TRUE) from the original CuffDiff output files.

Value

A CuffSet object. A ‘pointer’ class that allows interaction with cufflinks/cuffdiff data via a SQLite database backend.

Note

None.

Author(s)

Loyal A. Goff

References

None.

Examples

a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Read cufflinks data in sample directory and creates CuffSet object

---

repFpkm-methods

Retrieve FPKM values

Description

Returns a data.frame from @repFpkm slot

Details

Returns a data.frame of replicate FPKM values and associated statistics.

Value

A data.frame of replicate-level FPKM values and associated statistics for a set of features.

Methods

signature(object = "CuffData")
signature(object = "CuffFeature")
signature(object = "CuffFeatureSet"
replicates

Note
None

Author(s)
Loyal A. Goff

References
None

Examples
data(sampleData)
repFpkm(PINK1)

replicates Get replicate sample list from CuffData object

Description
Returns a list of replicate names from a CuffData or CuffFeatureSet object

Usage
## S4 method for signature 'CuffData'
replicates(object)

Arguments
object An object of class ('CuffSet', 'CuffData')

Details
None

Value
A list of replicate sample names

Note
None

Author(s)
Loyal A. Goff

References
None
runInfo

See Also
None

Examples

a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Create CuffSet object
replicates(a@genes)

runInfo

Retrieve run parameters and information from a CuffSet object

Description

Returns a data.frame of cuffdiff run parameters and information

Usage

## S4 method for signature 'CuffSet'
runInfo(object)

Arguments

object An object of class ('CuffSet')

Details

None

Value

A data.frame of run parameters

Note

None

Author(s)

Loyal A. Goff

References

None

See Also

None

Examples

a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Create CuffSet object
runInfo(a)
**sampleGeneSet**

---

**sampleGeneSet**

---

**Description**

A sample CuffGeneSet data set for 20 genes.

**Usage**

data(sampleData)

**Format**

sampleGeneSet is a CuffGeneSet (extends CuffFeatureSet) object containing all sample gene-, isoform-, TSS-, and CDS-level data for 20 different genes. These data were derived from a toy set of hESC-vs-iPSC-vs-Fibroblast RNA-Seq expression data.

**Details**

None

**Source**

None

**References**

None

**Examples**

data(sampleData)

---

**sampleIDs**

---

**Description**

A vector of gene_ids used to create 'sampleGeneSet' example

**Usage**

data(sampleData)

**Format**

The format is: chr "sampleIDs"
samples

Details
None

Source
None

References
None

Examples
data(sampleData)

samples  Get sample list from CuffData object

Description
Returns a list of sample names from a CuffData or CuffFeatureSet object

Usage
## S4 method for signature 'CuffData'
samples(object)

Arguments

object  An object of class ('CuffData', 'CuffFeatureSet', 'CuffFeature')

Details
None

Value
A list of sample names

Note
None

Author(s)
Loyal A. Goff

References
None
shannon.entropy

See Also
None

Examples

```r
a <- readCufflinks(system.file("extdata", package="cummeRbund")) # Create CuffSet object
samples(a@genes)
```

<table>
<thead>
<tr>
<th>shannon.entropy</th>
<th>Shannon entropy</th>
</tr>
</thead>
</table>

Description
Calculates the Shannon entropy for a probability distribution

Usage

```
shannon.entropy(p)
```

Arguments

- `p`: A vector of probabilities (must sum to ~1)

Details
None

Value
Returns a numeric value for the Shannon entropy of the supplied probability distribution

Note
None

Author(s)
Loyal A. Goff

References
None

Examples

```r
x <- sample(1:500, 50)
p <- x/sum(x)
shannon.entropy(p)
```
sigMatrix

Description

Returns a ggplot2 plot object representing a matrix of significant features. This is a useful synopsis of all significant pairwise comparisons within the dataset.

Usage

```r
## S4 method for signature 'CuffSet'
sigMatrix(object, alpha=0.05, level='genes', orderByDist=FALSE)
```

Arguments

- `object`: An object of class CuffSet.
- `alpha`: An alpha value by which to filter multiple-testing corrected q-values to determine significance.
- `level`: Feature level to be queried for significance (must be one of c('genes','isoforms','TSS','CDS')).
- `orderByDist`: Logical. If TRUE then samples are re-ordered based on JS-distance from one another (fairly useless unless you have a specific need for this).

Details

Creates a matrix plot to illustrate the number of significant features of type 'level' at a given alpha from a cuffdiff run.

Value

A ggplot2 plot object

Note

None

Author(s)

Loyal A. Goff

References

None

Examples

```r
a<-readCufflinks(system.file("extdata", package="cummeRbund")) #Create CuffSet object from sample data
d<-sigMatrix(a) #Create csDensity plot
d #Render plot
```
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