

Package ‘consensusDE’

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Type Package

Title RNA-seq analysis using multiple algorithms

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Description This package allows users to perform DE analysis using multiple algorithms. It seeks consensus from multiple methods. Currently it supports ``Voom'', ``EdgeR'' and ``DESeq''. It uses RUV-seq (optional) to remove batch effects.

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buildSummarized

Generate summarized Read File for DE analyses

Description

This function will create a summarized file, describing reads from RNA-seq experiments that overlap a set of transcript features. Transcript features can be described as a gtf formatted table that is imported, or using a txdb. This is designed to be straightforward and with minimised parameters for first pass batch RNA-seq analyses.

Usage

```
buildSummarized(sample_table = NULL, bam_dir = NULL, gtf = NULL,
  tx_db = NULL, mapping_mode = "Union", read_format = NULL,
  ignore_strand = FALSE, fragments = TRUE, summarized = NULL,
  output_log = NULL, filter = FALSE,
  BamFileList_yiedsize = NA_integer_, n_cores = 1,
  force_build = FALSE, verbose = FALSE)
```

Arguments

sample_table	A data.frame describing samples. For paired mode it must contain 3 columns, with the names "file", "group" and "pairs". The filename is the name in the directory supplied with the "bam_dir" parameter below. This is not required if an existing summarized file is provided. Default=NULL
bam_dir	Full path to location of bam files listed in the "file" column in the sample_table provided above. This is not required if an existing summarized file is provided. Default=NULL
gtf	Full path to a gtf file describing the transcript coordinates to map the RNA-seq reads to. GTF file is not required if providing a pre-computed summarized experiment file previously generated using buildSummarized() OR a tx_db object (below). Default = NULL
tx_db	An R txdb object. E.g. TxDb.Dmelanogaster.UCSC.dm3.ensGene. Default = NULL
mapping_mode	Options are "Union", "IntersectionStrict" and "IntersectionNotEmpty". see "mode" in ?summarizeOverlaps for explanation. Default = "Union"
read_format	Are the reads from single-end or paired-end data? Option are "paired" or "single". An option must be selected. Default = NULL
ignore_strand	Ignore strand when mapping reads? see "ignore_strand" in ?summarizeOverlaps for explanation. Default=FALSE
fragments	When mapping_mode="paired", include reads from pairs that do not map with their corresponding pair? see "fragments" in ?summarizeOverlaps for explanation. Default = TRUE
summarized	Full path to a summarized experiment file. If buildSummarized() has already been performed, the output summarized file, saved in "/output_log/se.R" can be used as the input (e.g. if filtering is to be done). Default = NULL
output_log	Full path to directory for output of log files and saved summarized experiment generated.

filter	Perform filtering of low count and missing data from the summarized experiment file? This uses default filtering via "filterByExpr". See ?filterByExpr for further information. Default=FALSE
BamFileList_yieldsizes	If running into memory problems. Set the number of lines to an integer value. See "yieldSize" description in ?BamFileList for an explanation.
n_cores	Number of cores to utilise for reading in Bam files. Use with caution as can create memory issues if BamFileList_yieldsizes is not parameterised. Default = 1
force_build	If the sample_table contains less than two replicates per group, force a summarizedExperiment object to be built. Otherwise buildSummarized will halt. Default = FALSE.
verbose	Verbosity ON/OFF. Default=FALSE

Value

A summarized experiment

Examples

```
## Extract summarized following example in the vignette
## The example below will return a summarized experiment
## tx_db is obtained from TxDb.Dmelanogaster.UCSC.dm3.ensGene library
library(TxDb.Dmelanogaster.UCSC.dm3.ensGene)
## bam files are obtained from the GenomicAlignments package

## 1. Build a sample table that lists files and groupings
## - obtain list of files
file_list <- list.files(system.file("extdata", package="GenomicAlignments"),
                        recursive = TRUE,
                        pattern = "*bam$",
                        full = TRUE)
bam_dir <- as.character(gsub(basename(file_list)[1], "", file_list[1]))

## - create a sample table to be used with buildSummarized() below
## must be comprised of a minimum of two columns, named "file" and "group",
sample_table <- data.frame("file" = basename(file_list),
                           "group" = c("treat", "untreat"))

summarized_dm3 <- buildSummarized(sample_table = sample_table,
                                bam_dir = bam_dir,
                                tx_db = TxDb.Dmelanogaster.UCSC.dm3.ensGene,
                                read_format = "paired",
                                force_build = TRUE)
```

Description

Wrappers for a series of plots to be used as diagnostics in RNA-seq analyses. Currently 10 plots are possible using this function: 1) Mapped reads, 2) Relative Log Expression (RLE), 3) Principle Component Analysis (PCA), 4) Residuals from a batch correction model, e.g. RUVseq, 5) Hierarchical clustering, 6) Density distributions, 7) Boxplots, 8) MA plots, 9) Volcano Plots and 10) P-value distribution plots. Plots 1 to 6 utilise a "SeqExpressionSet" object for extracting information to plot. Plots 8-10 utilised a simple list class, containing all the data.frames of each comparison performed. See descriptions of each in the parameter options below and for format specification. See vignette for more information and examples.

Usage

```
diag_plots(se_in = NULL, merged_in = NULL, write = FALSE,
  plot_dir = NULL, legend = TRUE, label = TRUE, name = NULL,
  mapped_reads = FALSE, rle = FALSE, pca = FALSE,
  residuals = FALSE, hclust = FALSE, density = FALSE,
  boxplot = FALSE, ma = FALSE, volcano = FALSE, p_dist = FALSE)
```

Arguments

se_in	A "SeqExpressionSet" object or "RangedSummarizedExperiment" generated using "buildSummarized()". If the input is a "SeqExpressionSet", ensure that it included groups to be analysed. E.g. accessible as "se_in\$group". Groupings are used to automate colouring of samples in unsupervised analyses. Default = NULL
merged_in	A data.frame that contains the merged results which are included in the outputs from multi_de_pairs(). These contain the outputs from the pair-wise comparisons which allows plotting of MA, Volcano and p-value distributions. Where the outputs of multi_de_pairs() are to be used as inputs into diag_plots(), use multi_de_pairs()\$merged as inputs. See example below. Default = NULL
write	Write the results to a pdf file? Options: TRUE, FALSE. This is to be used together with "plot_dir" and "write" parameters (below). Will report an error and halt if is TRUE and "plot_dir" and "write" are NULL. Default = FALSE
plot_dir	If "write" is TRUE, where to write the files to? The directory must already exist. E.g. "/path/to/my/pretty/plots/". Default = NULL
legend	Include legend in plots? Legend is based on group data in se_in. Options: TRUE, FALSE. Default = FALSE
label	Include point labels in plots? Points are based on ID column from merged_in. Options: TRUE, FALSE. Default = FALSE
name	If "write" is TRUE, what to name the plot? The file name will always be preceded with "QC_" and end in ".pdf". E.g. name="very_pretty_plots" will produce a file named "QC_very_pretty_plots.pdf" in "/path/to/my/pretty/plots/". Default = NULL
mapped_reads	Plot mapped reads per sample as a barchart. Requires se_in to be a "SeqExpressionSet" and utilise "group" meta-data for colouring. Options: TRUE, FALSE. Default = FALSE
rle	Plot Relative Log Expression (RLE) of samples for assessment of sample quality. See ?plotRLE for further details. Requires se_in to be a "SeqExpressionSet" and utilise "group" meta-data for colouring. Options: TRUE, FALSE. Default = FALSE

pca	Perform unsupervised Principle Component Analysis (PCA) and plot results. By default performs Singular Value Decomposition. Requires <code>se_in</code> to be a "SeqExpressionSet" and utilise "group" meta-data for colouring. Options: TRUE, FALSE. Default = FALSE
residuals	If RUV-seq has been applied to dataset, plot the residuals identified in the model. Only works for one set of residuals. Data is also accessible using <code>pData(se_in)\$W_1</code> . Requires <code>se_in</code> to be a "SeqExpressionSet" and utilise "group" meta-data for colouring. Options: TRUE, FALSE. Default = FALSE
hclust	Performs unsupervised hierarchical clustering of samples. Colours sample below plot according to group and numbered by inputs. Requires <code>se_in</code> to be a "SeqExpressionSet" and utilise "group" meta-data for colouring. Options: TRUE, FALSE. Default = FALSE
density	Plot density distributions of $\log_2(\text{count-per-million})$. Will automatically extract normalised counts over non-normalised counts is available in "SeqExpressionSet". Requires <code>se_in</code> to be a "SeqExpressionSet" and utilise "group" meta-data for colouring. Options: TRUE, FALSE. Default = FALSE
boxplot	Boxplot of density distributions of $\log_2(\text{count-per-million})$. Will automatically extract normalised counts over non-normalised counts is available in "SeqExpressionSet". Requires <code>se_in</code> to be a "SeqExpressionSet" and utilise "group" meta-data for colouring. Options: TRUE, FALSE. Default = FALSE
ma	Plot Mean versus. \log_2 Fold-Change of comparison. Requires a data.frame as input to "merged_in" with the following column names "ID", "AvExpr", "Log2FC" and "Adj_PVal". The data frame should be sorted, as the top 10 in the table are also plotted. Options: TRUE, FALSE. Default = FALSE
volcano	Volcano plot of \log_2 Fold-Change and significance of comparison. Requires a data.frame as input to "merged_in" with the following column names "ID", "AvExpr", "Log2FC" and "Adj_PVal". The data frame should be sorted, as the top 10 in the table are also plotted. Options: TRUE, FALSE. Default = FALSE
p_dist	P-value distribution plot. Requires a data.frame as input to "merged_in" with the following column names "ID", "AvExpr", "Log2FC" and "Adj_PVal". The data frame should be sorted, as the top 10 in the table are also plotted. Options: TRUE, FALSE. Default = FALSE

Value

Returns pretty plots

Examples

```
## Load the example data set and attach
## The example below will display a PCA plot before normalisation
library(airway)
data(airway)
## Name the groups of the data.
colData(airway)$group <- colData(airway)$dex
## Identify the file locations
colData(airway)$file <- rownames(colData(airway))
## Filter low count data:
airway_filter <- buildSummarized(summarized = airway,
                                filter = TRUE)
## for illustration, use random sample of 1000 transcripts
set.seed(1234)
```

```

airway_filter <- sample(airway_filter, 1000)
## The following is example code to perform a PCA plot
## see vignette for more details of displaying each plot
## diag_plots(se_in = airway_filter,
##            name = "airway example data",
##            pca = TRUE)

```

multi_de_pairs

Batch - multiDE analysis of many comparisons

Description

Given a summarized experiment generated using `buildSummarized()` this function will automatically perform differential expression (DE) analysis for all possible groups using 3 different methods 1) EdgeR, 2) Voom and 3) DEseq2. It will also output 10x diagnostic plots automatically, if the plotting options are selected (see `?diag_plots` for more details).

Usage

```

multi_de_pairs(summarized = NULL, paired = "unpaired",
               intercept = NULL, adjust_method = "BH", ruv_correct = FALSE,
               ensembl_annotate = NULL, plot_dir = NULL, output_voom = NULL,
               output_edger = NULL, output_deseq = NULL, output_combined = NULL,
               verbose = FALSE, legend = TRUE, label = TRUE)

```

Arguments

summarized	A "RangedSummarizedExperiment" object with included groups to be analysed. For format specifications see <code>?buildSummarized</code> . E.g. accessible as <code>"summarized\$group"</code> . Groups are used to automate colouring of samples in unsupervised analyses. Default = NULL
paired	Are the sample paired? If "paired" a paired statistical analysis by including factors as pairs described in the "pairs" column of the "RangedSummarizedExperiment" object in the model (accessible as <code>summarized\$pairs</code>). Options are "unpaired" or "paired". Default="unpaired"
intercept	Optional ability to set the base term for fitting the model. This is not necessary as all pairs are computed automatically. The base term, if set, must match the name of group in <code>"summarized\$group"</code> . Default = NULL
adjust_method	Method used for multiple comparison adjustment of p-values. Options are: "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr" or "none". See <code>?p.adjust.methods</code> for a details description and references. Default = "BH"
ruv_correct	Remove Unwanted Variation (RUV)? See <code>?RUVr</code> for description. Currently only RUVr, which used the residuals is enabled and one factor of variation is determined. If set to TRUE and a "plot_dir" is provided, additional plots after RUV correction and the RUV residuals will be reported. Residuals are obtained through fitting a generalised linear model (GLM) using EdgeR. Residuals are then incorporated into the SummarizedExperiment object and models for DE analysis. Options = TRUE, FALSE. Default = FALSE.

ensembl_annotate	If the dataset has been mapped to ensembl transcript identifiers, obtain additional annotation of the ensembl transcripts. A R Genome Wide Annotation object e.g. org.Mm.eg.db for mouse or org.Hs.eg.db for human must be provided. Default = NULL
plot_dir	Full path to directory for output of plots (pdf files). See ?diag_plots for more details. Default = NULL
output_voom	If you wish to output the results of the Voom analysis, provide a full path to directory for output of files. Default = NULL
output_edger	If you wish to output the results of the EdgeR analysis, provide a full path to directory for output of files. Default = NULL
output_deseq	If you wish to output the results of the DEseq2 analysis, provide a full path to directory for output of files. Default = NULL
output_combined	consensusDE will report the results of Voom, EdgeR and DEseq2 as a combined report. If you wish to output the results of the COMBINED analysis, provide a full path to directory for output of files. In addition to the combined data, it will also output the raw count and normalised data to the same directory. Default = NULL
verbose	Verbosity ON/OFF. Default=FALSE
legend	Include legend in plots? Legend is based on group data in summarized Options: TRUE, FALSE. Default = TRUE
label	Include point labels in plots? Points are based on ID column after DE analysis from merged results. Options: TRUE, FALSE. Default = TRUE

Value

A list of all the comparisons conducted. ## See vignette for more details.

Examples

```
## Load the example data set and attach - see vignette for more details
## The example below will perform DE analysis on all pairs of data
library(airway)
data(airway)
## Name groups of the data.
colData(airway)$group <- colData(airway)$dex
## Identify file locations
colData(airway)$file <- rownames(colData(airway))
#' ## Filter low count data:
airway_filter <- buildSummarized(summarized = airway,
                                filter = TRUE)
## for illustration, we only use random sample of 1000 transcripts
set.seed(1234)
airway_filter <- sample(airway_filter, 1000)
## Run multi_de_pairs() with-out RUV correction
## To run with RUV correction, use ruv_correct = TRUE
all_pairs_airway <- multi_de_pairs(summarized = airway_filter,
                                ruv_correct = FALSE,
                                paired = "unpaired")
```

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