Package 'fishpond'

November 12, 2026

Title Fishpond: downstream methods and tools for expression data

Version 2.18.0

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Description Fishpond contains methods for differential transcript and gene expression analysis of RNA-seq data using inferential replicates for uncertainty of abundance quantification, as generated by Gibbs sampling or bootstrap sampling. Also the package contains a number of utilities for working with Salmon and Alevin quantification files.

Imports graphics, stats, utils, methods, abind, gtools, qvalue, S4Vectors, IRanges, SummarizedExperiment, GenomicRanges, matrixStats, svMisc, Matrix, SingleCellExperiment, jsonlite

Suggests testthat, knitr, rmarkdown, macrophage, tximeta, org.Hs.eg.db, samr, DESeq2, apeglm, tximportData, limma, ensembldb, EnsDb.Hsapiens.v86, GenomicFeatures, AnnotationDbi, pheatmap, Gviz, GenomeInfoDb, data.table

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Encoding UTF-8

URL https://thelovelab.github.io/fishpond, https://thelovelab.com/mikelove/fishpond

BugReports https://support.bioconductor.org/tag/fishpond

biocViews Sequencing, RNASeq, GeneExpression, Transcription, Normalization, Regression, MultipleComparison, BatchEffect, Visualization, DifferentialExpression, DifferentialSplicing, AlternativeSplicing, SingleCell

VignetteBuilder knitr

LazyData true

RoxygenNote 7.2.3

git_url https://git.bioconductor.org/packages/fishpond

git_branch RELEASE_3_23

git_last_commit d572667

git_last_commit_date 2026-04-28

Repository Bioconductor 3.23

Date/Publication 2026-11-11

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`fishpond-package` *Fishpond: downstream methods and tools for expression data*

Description

This package provides statistical methods and other tools for working with Salmon and Alevin quantification of RNA-seq data. Fishpond contains the Swish non-parametric method for detecting differential transcript expression (DTE). Swish can also be used to detect differential gene expresion (DGE), to perform allelic analysis, or to assess changes in isoform proportions.

Details

The main Swish functions are:

- `scaleInfReps` - scaling transcript or gene expression data
- `labelKeep` - labelling which features have sufficient counts
- `swish` - perform non-parametric differential analysis
- Plots, e.g., `plotMASwish`, `plotInfReps`

All software-related questions should be posted to the Bioconductor Support Site:

https://support.bioconductor.org

The code can be viewed at the GitHub repository, which also lists the contributor code of conduct:

https://github.com/mikelove/fishpond

References

Swish method:

Zhu, A., Srivastava, A., Ibrahim, J.G., Patro, R., Love, M.I. (2019) Nonparametric expression analysis using inferential replicate counts. Nucleic Acids Research. https://doi.org/10.1093/nar/gkz622

Compression, makeInfReps and splitSwish:

Van Buren, S., Sarkar, H., Srivastava, A., Rashid, N.U., Patro, R., Love, M.I. (2020) Compression of quantification uncertainty for scRNA-seq counts. bioRxiv. https://doi.org/10.1101/2020.07.06.189639

addStatsFromCSV *Read statistics and nulls from CSV file*

Description

After running splitSwish and the associated Snakefile, this function can be used to gather and add the results to the original object. See the alevin section of the vignette for an example.

Usage

```
addStatsFromCSV(y = NULL, infile, estPi0 = FALSE)
```

Arguments

y	a SummarizedExperiment (if NULL, function will output a data.frame)
infile	character, path to the summary.csv file
estPi0	logical, see swish

Value

the SummarizedExperiment with metadata columns added, or if y is NULL, a data.frame of compiled results

alevinEC *Construct a sparse matrix of transcript compatibility counts from alevin output*

Description

Constructs a UMI count matrix with equivalence class identifiers in the rows and barcode identifiers in the columns. The count matrix is generated from one or multiple 'bfh.txt' files that have been created by running alevin-fry with the –dumpBFH flag. Alevin-fry - https://doi.org/10.1186/s13059-019-1670-y

Usage

```
alevinEC(
  paths,
  tx2gene,
  multigene = FALSE,
  ignoreTxVersion = FALSE,
  ignoreAfterBar = FALSE,
  quiet = FALSE
)
```

Arguments

paths	'Charachter' or 'character vector', path specifying the location of the 'bfh.txt' files generated with alevin-fry.
tx2gene	A 'dataframe' linking transcript identifiers to their corresponding gene identifiers. Transcript identifiers must be in a column 'isoform_id'. Corresponding gene identifiers must be in a column 'gene_id'.
multigene	'Logical', should equivalence classes that are compatible with multiple genes be retained? Default is 'FALSE', removing such ambiguous equivalence classes.
ignoreTxVersion	logical, whether to split the isoform id on the '.' character to remove version information to facilitate matching with the isoform id in 'tx2gene' (default FALSE).
ignoreAfterBar	logical, whether to split the isoform id on the '|' character to facilitate matching with the isoform id in 'tx2gene' (default FALSE).
quiet	'Logical', set 'TRUE' to avoid displaying messages.

Value

A list with two elements. The first element 'counts' is a sparse count matrix with equivalence class identifiers in the rows and barcode identifiers followed by an underscore and a sample identifier in the columns. The second element 'tx2gene_matched' allows for linking the equivalence class identifiers to their respective transcripts and genes.

Details

The resulting count matrix uses equivalence class identifiers as rownames. These can be linked to respective transcripts and genes using the 'tx2gene_matched' element of the output. Specifically, if the equivalence class identifier reads 1|2|8, then the equivalence class is compatible with the transcripts and their respective genes in rows 1, 2 and 8 of 'tx2gene_matched'.

Author(s)

Jeroen Gilis

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computeInfRV *Compute inferential relative variance (InfRV)*

Description

`InfRV` is a useful quantity for comparing groups of features (transcripts, genes, etc.) by inferential uncertainty. This function provides computation of the mean InfRV over samples, per feature, stored in `mcols(y)$meanInfRV`.

Usage

```
computeInfRV(y, pc = 5, shift = 0.01, meanVariance, useCounts = FALSE)
```

Arguments

y	a SummarizedExperiment
pc	a pseudocount parameter for the denominator
shift	a final shift parameter
meanVariance	logical, use pre-computed inferential mean and variance assays instead of counts and computed variance from infReps. If missing, will use pre-computed mean and variance when present
useCounts	logical, whether to use the MLE count matrix for the mean instead of mean of inferential replicates. this argument is for backwards compatability, as previous versions used counts. Default is FALSE

Details

InfRV is defined in Zhu et al. (2019) as: $\max(s^2 - \mu, 0)/\mu$, using the inferential sample variance and sample mean. This formulation takes the non-Poisson part of the inferential variance and scales by the mean, which effectively stabilizes inferential uncertainty over mean count. In practice, we also add pc to the denominator and shift to the final quantity, to facilitate visualization.

This function also computes and adds the mean and variance of inferential replicates, which can be useful ahead of plotInfReps. Note that InfRV is not used in the swish statistical method (for generating test statistics, p-values or q-values), it is just for visualization.

Value

a SummarizedExperiment with meanInfRV in the metadata columns

References

Anqi Zhu, Avi Srivastava, Joseph G Ibrahim, Rob Patro, Michael I Love "Nonparametric expression analysis using inferential replicate counts" Nucleic Acids Research (2019). https://doi.org/10.1093/nar/gkz622

---

deswish *deswish: DESeq2-apeglm With Inferential Samples Helps*

Description

The DESeq2-apeglm With Inferential Samples implementation supposes a hierarchical distribution of log2 fold changes. The final posterior standard deviation is calculated by adding the posterior variance from modeling biological replicates computed by apeglm, and the observed variance on the posterior mode over inferential replicates. This function requires the DESeq2 and apeglm packages to be installed and will print an error if they are not found.

Usage

```
deswish(y, x, coef)
```

Arguments

y	a SummarizedExperiment containing the inferential replicate matrices, as output by `tximeta`, and then with `labelKeep` applied. One does not need to run `scaleInfReps` as scaling is done internally via DESeq2.
x	the design matrix
coef	the coefficient to test (see `lfcShrink`)

Value

a SummarizedExperiment with metadata columns added: the log2 fold change and posterior SD using inferential replicates, and the original log2 fold change (apeglm) and its posterior SD

References

The `DESeq` and `lfcShrink` function in the `DESeq2` package:

Zhu, Ibrahim, Love "Heavy-tailed prior distributions for sequence count data: removing the noise and preserving large differences" Bioinformatics (2018).

Love, Huber, Anders "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2" Genome Biology (2014).

Examples

```
# a small example... 500 genes, 10 inf reps
y <- makeSimSwishData(m=500, numReps=10)
y <- labelKeep(y)
#y <- deswish(y, ~condition, "condition_2_vs_1")
```

`getTrace` *Obtain a trace of inferential replicates for a sample*

Description

Simple helper function to obtain a trace (e.g. MCMC trace) of the ordered inferential replicates for one samples. Supports either multiple features, `idx`, or multiple samples, `samp_idx` (not both). Returns a tidy data.frame for easy plotting.

Usage

```
getTrace(y, idx, samp_idx)
```

Arguments

y	a SummarizedExperiment with inferential replicates as assays `infRep1` etc.
idx	the names or row numbers of the gene or transcript to plot
samp_idx	the names or column numbers of the samples to plot

Value

a data.frame with the counts along the interential replicates, possible with additional columns specifying feature or sample

Examples

```
y <- makeSimSwishData()
getTrace(y, "gene-1", "s1")
```

`importAllelicCounts` *Import allelic counts as a SummarizedExperiment*

Description

Read in Salmon quantification of allelic counts from a diploid transcriptome. Assumes that diploid transcripts are marked with the following suffix: an underscore and a consistent symbol for each of the two alleles, e.g. `ENST123_M` and `ENST123_P`, or `ENST123_alt` and `ENST123_ref`, etc. `importAllelicCounts` requires the tximeta package. Further information in Details below.

Usage

```
importAllelicCounts(
  coldata,
  a1,
  a2,
  format = c("wide", "assays"),
  tx2gene = NULL,
  ...
)
```

Arguments

`coldata`	a data.frame as used in `tximeta`
`a1`	the symbol for the effect allele
`a2`	the symbol for the non-effect allele
`format`	either `"wide"` or `"assays"` for whether to combine the allelic counts as columns (wide) or put the allelic count information in different assay slots (assays). For wide output, the data for the non-effect allele (a2) comes first, then the effect allele (a1), e.g. `[a2 | a1]`. The `ref` level of the factor variable `se$allele` will be `"a2"` (so by default comparisons will be: a1 vs a2). For assays output, all of the original matrices are renamed with a prefix, either `a1-` or `a2-`.
`tx2gene`	optional, a data.frame with first column indicating transcripts, second column indicating genes (or any other transcript grouping). Alternatively, this can be a GRanges object with required columns `tx_id`, and `group_id` (see `makeTx2Tss`). For more information on this argument, see Details.
`...`	any arguments to pass to tximeta

Details

Requirements - There must be exactly two alleles for each transcript, and the `--keep-duplicates` option should be used in Salmon indexing to avoid removal of transcripts with identical sequence. The output object has half the number of transcripts, with the two alleles either stored in a `"wide"` object, or as re-named `"assays"`. Note carefully that the symbol provided to `a1` is used as the effect allele, and `a2` is used as the non-effect allele (see the `format` argument description and Value description below).

tx2gene - The two columns should include the `a1` and `a2` suffix for the transcripts and genes/groups, or those will be added internally, if it is detected that the first transcript does not have these suffices. For example if `_alt` or `_ref`, or `_M` or `_P` (as indicated by the `a1` and `a2` arguments) are not present in the table, the table rows will be duplicated with those suffices added on behalf of the user. If `tx2gene` is not provided, the output object will be transcript-level. Do not attempt to set the `txOut` argument, it will conflict with internal calls to downstream functions. If the a1/a2 suffices are not at the end of the transcript name in the quantification files, e.g. `ENST123_M|<metadata>`, then `ignoreAfterBar=TRUE` can be used to match regardless of the string following `|` in the quantification files.

`skipMeta=TRUE` is used, as it is assumed the diploid transcriptome does not match any reference transcript collection. This may change in future iterations of the function, depending on developments in upstream annotations and software.

If `tx2gene` is a GRanges object, the rowRanges of the output will be the reduced ranges of the grouped input ranges, with `tx_id` collapsed into a CharacterList, and TSS positions saved as an IntegerList, if these are not equal among the transcripts of a group. Other metadata columns are not manipulated, just the metadata for the first range is returned.

Value

a SummarizedExperiment, with allele counts (and other data) combined into a wide matrix `[a2 | a1]`, or as assays (a1, then a2). The original strings associated with a1 and a2 are stored in the metadata of the object, in the `alleles` list element. Note the reference level of `se$allele` will be `"a2"`, such that comparisons by default will be a1 vs a2 (effect vs non-effect).

References

Euphy Wu, Noor P. Singh, Kwangbom Choi, Mohsen Zakeri, Matthew Vincent, Gary A. Churchill, Cheryl L. Ackert-Bicknell, Rob Patro, Michael I. Love. "Detecting isoform-level allelic imbalance accounting for inferential uncertainty" bioRxiv (2022) https://doi.org/10.1101/2022.08.12.503785

`isoformProportions` *Create isoform proportions from scaled data*

Description

Takes output of scaled (and optionally filtered) counts and returns isoform proportions by dividing out the total scaled count for the gene for each sample. The operation is performed on the `counts` assay, then creating a new assay called `isoProp`, and on all of the inferential replicates, turning them from counts into isoform proportions. Any transcripts (rows) from single isoform genes are removed, and the transcripts will be re-ordered by gene ID.

Usage

```
isoformProportions(y, geneCol = "gene_id", quiet = FALSE)
```

Arguments

`y`	a SummarizedExperiment
`geneCol`	the name of the gene ID column in the metadata columns for the rows of `y`
`quiet`	display no messages

Value

a SummarizedExperiment, with single-isoform transcripts removed, and transcripts now ordered by gene

---

`labelKeep` *Label rows to keep based on minimal count*

Description

Adds a column `keep` to `mcols(y)` that specifies which rows of the SummarizedExperiment will be included in statistical testing. Rows are not removed, just marked with the logical `keep`.

Usage

```
labelKeep(y, minCount = 10, minN = 3, x)
```

Arguments

`y`	a SummarizedExperiment
`minCount`	the minimum count
`minN`	the minimum sample size at `minCount`
`x`	the name of the condition variable, will use the smaller of the two groups to set `minN`. Similar to edgeR's `filterByExpr`, as the smaller group grows past 10, `minN` grows only by 0.7 increments of sample size

Value

a SummarizedExperiment with a new column `keep` in `mcols(y)`

Examples

```
y <- makeSimSwishData()
y <- scaleInfReps(y)
y <- labelKeep(y)
```

`loadFry` *Load in data from alevin-fry USA mode*

Description

Enables easy loading of sparse data matrices provided by alevin-fry USA mode.

Usage

```
loadFry(fryDir, outputFormat = "scRNA", nonzero = FALSE, quiet = FALSE)
```

Arguments

`fryDir`	path to the output directory returned by alevin-fry quant command. This directory should contain a `metainfo.json`, and an alevin folder which contains `quants_mat.mtx`, `quants_mat_cols.txt` and `quants_mat_rows.txt`
`outputFormat`	can be *either* be a list that defines the desired format of the output `SingleCellExperiment` object *or* a string that represents one of the pre-defined output formats, which are "scRNA", "snRNA", "all", "scVelo", "velocity", "U+S+A" and "S+A". See details for the explanations of the pre-defined formats and how to define custom format.
`nonzero`	whether to filter cells with non-zero expression value across all genes (default `FALSE`). If `TRUE`, this will filter based on all assays. If a string vector of assay names, it will filter based on the matching assays in the vector. If not in USA mode, it must be TRUE/FALSE/counts.
`quiet`	logical whether to display no messages

Value

A `SingleCellExperiment` object that contains one or more assays. Each assay consists of a gene by cell count matrix. The row names are feature names, and the column names are cell barcodes

Details about `loadFry`

This function consumes the result folder returned by running alevin-fry quant in unspliced, spliced, ambiguous (USA) quantification mode, and returns a `SingleCellExperiment` object that contains a final count for each gene within each cell. In USA mode, alevin-fry quant returns a count matrix contains three types of count for each feature (gene) within each sample (cell or nucleus), which represent the spliced mRNA count of the gene (S), the unspliced mRNA count of the gene (U), and the count of UMIs whose splicing status is ambiguous for the gene (A). For each assay defined by

outputFormat, these three counts of a gene within a cell will be summed to get the final count of the gene according to the rule defined in the outputFormat. The returned object will contains the desired assays defined by outputFormat, with rownames as the barcode of samples and colnames as the feature names.

Details about the output format

The outputFormat argument takes *either* be a list that defines the desired format of the output SingleCellExperiment object *or* a string that represents one of the pre-defined output format.

Currently the pre-defined formats of the output SingleCellExperiment object are:

"scRNA": This format is recommended for single cell experiments. It returns a counts assay that contains the S+A count of each gene in each cell, and a unspliced assay that contains the U count of each gene in each cell.

"snRNA", "all" and "U+S+A": These three formats are the same. They return a counts assay that contains the U+S+A count of each gene in each cell without any extra layers. "snRNA" is recommended for single-nucleus RNA-sequencing experiments. "raw" is recommended for mimicing CellRanger 7's behavior, which returns this format for both single-cell and single-nucleus experiments.

"S+A": This format returns a counts assay that contains the S+A count of each gene in each cell.

"raw": This format puts the three kinds of counts into three separate assays, which are unspliced, spliced and ambiguous.

"velocity": This format contains two assays. The spliced assay contains the S+A count of each gene in each cell. The unspliced assay contains the U counts of each gene in each cell.

"scVelo": This format is for direct entry into velociraptor R package or other scVelo downstream analysis pipeline for velocity analysis in R with Bioconductor. It adds the expected "S"-pliced assay and removes errors for size factors being non-positive.

A custom output format can be defined using a list. Each element in the list defines an assay in the output SingleCellExperiment object. The name of an element in the list will be the name of the corresponding assay in the output object. Each element in the list should be defined as a vector that takes at least one of the three kinds of count, which are U, S and A. See the provided toy example for defining a custom output format.

Author(s)

Dongze He, with contributions from Steve Lianoglou, Wes Wilson

References

alevin-fry publication:

He, D., Zakeri, M., Sarkar, H. et al. "Alevin-fry unlocks rapid, accurate and memory-frugal quantification of single-cell RNA-seq data." Nature Methods 19, 316–322 (2022). https://doi.org/10.1038/s41592-022-01408-3

Examples

```
# Get path for minimal example avelin-fry output dir
testdat <- fishpond:::readExampleFryData("fry-usa-basic")

# This is exactly how the velocity format defined internally.
custom_velocity_format <- list("spliced"=c("S","A"), "unspliced"=c("U"))

# Load alevin-fry gene quantification in velocity format
sce <- loadFry(fryDir=testdat$parent_dir, outputFormat=custom_velocity_format)
SummarizedExperiment::assayNames(sce)

# Load the same data but use pre-defined, velociraptor R pckage desired format
scvelo_format <- "scVelo"

scev <- loadFry(fryDir=testdat$parent_dir, outputFormat=scvelo_format, nonzero=TRUE)
SummarizedExperiment::assayNames(scev)
```

`makeInfReps` *Make pseudo-inferential replicates from mean and variance*

Description

Makes pseudo-inferential replicate counts from `mean` and `variance` assays. The simulated counts are drawn from a negative binomial distribution, with `mu=mean` and `size` set using a method of moments estimator for dispersion.

Usage

```
makeInfReps(y, numReps, minDisp = 0.001)
```

Arguments

`y`	a SummarizedExperiment
`numReps`	how many inferential replicates
`minDisp`	the minimal dispersion value, set after method of moments estimation from inferential mean and variance

Details

Note that these simulated counts only reflect marginal variance (one transcript or gene at a time), and do not capture the covariance of counts across transcripts or genes, unlike imported inferential replicate data. Therefore, `makeInfReps` should not be used with `summarizeToGene` to create gene-level inferential replicates if inferential replicates were originally created on the transcript level. Instead, import the original inferential replicates.

Value

a SummarizedExperiment

References

Van Buren, S., Sarkar, H., Srivastava, A., Rashid, N.U., Patro, R., Love, M.I. (2020) Compression of quantification uncertainty for scRNA-seq counts. bioRxiv. https://doi.org/10.1101/2020.07.06.189639

Examples

```
library(SummarizedExperiment)
mean <- matrix(1:4,ncol=2)
variance <- mean
se <- SummarizedExperiment(list(mean=mean, variance=variance))
se <- makeInfReps(se, numReps=50)
```

`makeSimSwishData` *Make simulated data for swish for examples/testing*

Description

Makes a small swish dataset for examples and testing. The first six genes have some differential expression evidence in the counts, with varying degree of inferential variance across inferential replicates (1-2: minor, 3-4: some, 5-6: substantial). The 7th and 8th genes have all zeros to demonstrate `labelKeep`.

Usage

```
makeSimSwishData(
  m = 1000,
  n = 10,
  numReps = 20,
  null = FALSE,
  meanVariance = FALSE,
  allelic = FALSE,
  diffAI = FALSE,
  dynamicAI = FALSE
)
```

Arguments

`m`	number of genes
`n`	number of samples
`numReps`	how many inferential replicates to generate
`null`	logical, whether to make an all null dataset
`meanVariance`	logical, whether to output only mean and variance of inferential replicates
`allelic`	logical, whether to make an allelic sim dataset
`diffAI`	logical, whether to make a differential allelic sim dataset
`dynamicAI`	logical, whether to make a dynamic allelic sim dataset

Value

a SummarizedExperiment

Examples

```
library(SummarizedExperiment)
y <- makeSimSwishData()
assayNames(y)
```

`makeTx2Tss` *Make a GRanges linking transcripts to TSS within gene*

Description

This helper function takes either a TxDb/EnsDb or GRanges object as input and outputs a GRanges object where transcripts are aggregated to the gene + TSS (transcription start site). For nearby TSS that should be grouped together, see `maxgap`.

Usage

```
makeTx2Tss(x, maxgap = 0)
```

Arguments

`x`	either TxDb/EnsDb or GRanges object. The GRanges object should have metadata columns `tx_id` and `gene_id`
`maxgap`	integer, number of basepairs to use determining whether to combine nearby TSS

Value

GRanges with columns `tx_id`, `tss`, and `group_id`

Examples

```
## Not run:
library(EnsDb.Hsapiens.v86)
edb <- EnsDb.Hsapiens.v86
t2t <- makeTx2Tss(edb)

## End(Not run)
```

`miniSwish` *Helper function for distributing Swish on a subset of data*

Description

This function is called by the `Snakefile` that is generated by `splitSwish`. See alevin example in the vignette. As such, it doesn't need to be run by users in an interactive R session.

Usage

```
miniSwish(
  infile,
  outfile,
  numReps = 20,
  lengthCorrect = FALSE,
  overwrite = FALSE,
  ...
)
```

Arguments

`infile`	path to an RDS file of a SummarizedExperiment
`outfile`	a CSV file to write out
`numReps`	how many inferential replicates to generate
`lengthCorrect`	logical, see `scaleInfReps`, and Swish vignette. As this function is primarily for alevin, the default is `FALSE`
`overwrite`	logical, whether `outfile` should overwrite an existing file
`...`	arguments passed to `swish`

Details

Note that the default for length correction is FALSE, as opposed to the default in `scaleInfReps` which is TRUE. The default for `numReps` here is 20.

Value

nothing, files are written out

plotAllelicGene *Plot allelic counts in a gene context using Gviz*

Description

Plot allelic data (allelic proportions, isoform propostions) in a gene context leveraging the Gviz package. See the allelic vignette for example usage. TPM and count filters are used by default to clean up the plot of features with minimal signal; note that the isoform proportion displayed at the bottom of the plot is among the features that pass the filtering steps. If the function is not responding, it is likely due to issues connecting to UCSC servers to draw the ideogram, in this case set `ideogram=FALSE`.

Usage

```
plotAllelicGene(
  y,
  gene,
  db,
  region = NULL,
  symbol = NULL,
  genome = NULL,
  tpmFilter = 1,
  isoPropFilter = 0.05,
  countFilter = 10,
  pc = 1,
  transcriptAnnotation = "symbol",
  labels = list(a2 = "a2", a1 = "a1"),
  qvalue = TRUE,
  log2FC = TRUE,
  ideogram = FALSE,
  cov = NULL,
  covFacetIsoform = FALSE,
  allelicCol = c("dodgerblue", "goldenrod1"),
  isoformCol = "firebrick",
  statCol = "black",
  gridCol = "grey80",
  baselineCol = "black",
  titleCol = "black",
  titleAxisCol = "black",
  titleBgCol = "white",
  geneBorderCol = "darkblue",
  geneFillCol = "darkblue",
  genomeAxisCol = "black",
  innerFontCol = "black",
  ...
)
```

Arguments

y	a SummarizedExperiment (see swish)
gene	the name of the gene of interest, requires a column gene_id in the metadata columns of the rowRanges of y
db	either a TxDb or EnsDb object to use for the gene model
region	GRanges, the region to be displayed in the Gviz plot. if not specified, will be set according to the gene plus 20 of the total gene extent on either side
symbol	alternative to gene, to specify the gene of interest according to a column symbol in the metadata columns of the rowRanges of y
genome	UCSC genome code (e.g. "hg38", if not specified it will use the GenomeInfoDb::genome() of the rowRanges of y
tpmFilter	minimum TPM value (mean over samples) to keep a feature
isoPropFilter	minimum percent of isoform proportion to keep a feature
countFilter	minimum count value (mean over samples) to keep a feature
pc	pseudocount to avoid dividing by zero in allelic proportion calculation
transcriptAnnotation	argument passed to Gviz::GeneRegionTrack ("symbol", "gene", "transcript", etc.)
labels	list, labels for a2 (non-effect) and a1 (effect) alleles
qvalue	logical, whether to inclue qvalue track
log2FC	logical, whether to include log2FC track
ideogram	logical, whether to include ideogram track
cov	character specifying a factor or integer variable to use to facet the allelic proportion plots, should be a column in colData(y)
covFacetIsoform	logical, if cov is provided, should it also be used to facet the isoform proportion track, in addition to the allelic proportion track
allelicCol	the colors of the points and lines for allelic proportion
isoformCol	the colors of the points and lines for isoform proportion
statCol	the color of the lollipops for q-value and log2FC
gridCol	the color of the grid in the data tracks
baselineCol	the color of the horizontal baseline for q-value and lo2gFC
titleCol	font color of the side titles (track labels)
titleAxisCol	axis color of the side titles (track labels)
titleBgCol	background color of the side titles (track labels)
geneBorderCol	the color of the borders and font in gene region track
geneFillCol	the color of the fill in the gene region track
genomeAxisCol	line color of the genome axis track
innerFontCol	font color of genome axis track, ideogram, and allelic proportion legend
...	additional arguments passed to Gviz::plotTracks()

Value

nothing, a plot is displayed

References

The methods for allelic expression analysis are described in:

Euphy Wu, Noor P. Singh, Kwangbom Choi, Mohsen Zakeri, Matthew Vincent, Gary A. Churchill, Cheryl L. Ackert-Bicknell, Rob Patro, Michael I. Love. "Detecting isoform-level allelic imbalance accounting for inferential uncertainty" bioRxiv (2022) https://doi.org/10.1101/2022.08.12.503785

This function makes use of the Gviz package that is described in:

Hahne, F., Ivanek, R. (2016). Visualizing Genomic Data Using Gviz and Bioconductor. In: Mathé, E., Davis, S. (eds) Statistical Genomics. Methods in Molecular Biology, vol 1418. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-3578-9_16

---

plotAllelicHeatmap *Plot allelic ratio heatmap*

Description

Plot allelic ratio heatmap over features and samples using the pheatmap package. The a1/(a2 + a1) ratio is displayed.

Usage

```
plotAllelicHeatmap(
  y,
  idx,
  breaks = NULL,
  cluster_cols = FALSE,
  main = "Allelic ratio",
  stripAfterChar = "-",
  ...
)
```

Arguments

y	a SummarizedExperiment (see swish)
idx	a numeric or logical vector of which features to plot
breaks	breaks passed along to pheatmap
cluster_cols	logical, passed to pheatmap
main	title of the plot
stripAfterChar	for the column names, if specified will strip allelic identifiers after this character, default is hyphen. set to NULL to avoid this action
...	other arguments passed to pheatmap

Value

nothing, a plot is displayed

References

The methods for allelic expression analysis are described in:

Euphy Wu, Noor P. Singh, Kwangbom Choi, Mohsen Zakeri, Matthew Vincent, Gary A. Churchill, Cheryl L. Ackert-Bicknell, Rob Patro, Michael I. Love. "Detecting isoform-level allelic imbalance accounting for inferential uncertainty" bioRxiv (2022) https://doi.org/10.1101/2022.08.12.503785

This function makes use of the pheatmap package:

Kolde, Raivo. "Pheatmap: pretty heatmaps." R package version 1.2 (2012): 726.

`plotInfReps` *Plot inferential replicates for a gene or transcript*

Description

For datasets with inferential replicates, boxplots are drawn for the two groups and potentially grouped by covariates. For datasets with only mean and variance, points and intervals (95 approximation) are drawn. Additionally, for numeric `x` values, points and intervals will be drawn and `computeInfRV` should be run first in order to add the mean and variance statistics.

Usage

```
plotInfReps(
  y,
  idx,
  x,
  cov = NULL,
 colsDrk = c("dodgerblue", "goldenrod4", "royalblue4", "red3", "purple4", "darkgreen"),
  colsLgt = c("lightblue1", "goldenrod1", "royalblue1", "salmon1", "orchid1",
    "limegreen"),
  xaxis,
  xlab,
  ylim,
  main,
  mainCol,
  legend = FALSE,
  legendPos = "topleft",
  legendTitle = FALSE,
  legendCex = 1,
  useMean = TRUE,
  q = qnorm(0.975),
  applySF = FALSE,
  reorder,
```

```
  thin,
  shiftX
)
```

Arguments

y	a SummarizedExperiment (see swish)
idx	the name or row number of the gene or transcript
x	the name of the condition variable for splitting and coloring the samples or cells. Also can be a numeric, e.g. pseudotime, in which case, cov can be used to designate groups for coloring
cov	the name of the covariate for adjustment
colsDrk	dark colors for the lines of the boxes
colsLgt	light colors for the inside of the boxes
xaxis	logical, whether to label the sample numbers. default is TRUE if there are less than 30 samples
xlab	the x-axis label
ylim	y limits
main	title
mainCol	name of metadata column to use for title (instead of rowname)
legend	logical, show simple legend (default FALSE)
legendPos	character, position of the legend (default "topleft")
legendTitle	logical, whether to add the name of the grouping variable as a title on the legend (default FALSE)
legendCex	numeric, size of the legend (default 1)
useMean	logical, when inferential replicates are not present or when x is continuous, whether to use the mean assay or the counts assay for plotting
q	numeric, the quantile to use when plotting the intervals when inferential replicates are not present or when x is continuous. Default is qnorm(.975) ~= 1.96 corresponding to 95 intervals
applySF	logical, when inferential replicates are not present, should y$sizeFactor be divided out from the mean and interval plots (default FALSE)
reorder	logical, should points within a group defined by condition and covariate be reordered by their count value (default is FALSE, except for alevin data)
thin	integer, should the mean and interval lines be drawn thin (the default switches from 0 [not thin] to 1 [thinner] at n=150 cells, and from 1 [thinner] to 2 [thinnest] at n=400 cells)
shiftX	when x is continuous and cov is provided, the amount to shift the values on the x-axis to improve visibility of the point and line ranges (will be subtracted from the first level of cov and added to the second level of cov)

Value

nothing, a plot is displayed

Examples

```
y <- makeSimSwishData()
plotInfReps(y, 3, "condition")

y <- makeSimSwishData(n=40)
y$batch <- factor(rep(c(1,2,3,1,2,3),c(5,10,5,5,10,5)))
plotInfReps(y, 3, "condition", "batch")
```

`plotMASwish` *MA plot - log fold change over average counts*

Description

MA plot - log fold change over average counts

Usage

```
plotMASwish(y, alpha = 0.05, sigcolor = "blue", ...)
```

Arguments

`y`	a SummarizedExperiment (see `swish`)
`alpha`	the FDR threshold for coloring points
`sigcolor`	the color for the significant points
`...`	passed to plot

Value

nothing, a plot is displayed

Examples

```
y <- makeSimSwishData()
y <- scaleInfReps(y)
y <- labelKeep(y)
y <- swish(y, x="condition")
plotMASwish(y)
```

salmonEC *Construct a sparse matrix of transcript compatibility counts from salmon output*

Description

Constructs a count matrix with equivalence class identifiers in the rows. The count matrix is generated from one or multiple 'eq_classes.txt' files that have been created by running salmon with the –dumpEq flag. Salmon - https://doi.org/10.1038/nmeth.4197

Usage

```
salmonEC(
  paths,
  tx2gene,
  multigene = FALSE,
  ignoreTxVersion = FALSE,
  ignoreAfterBar = FALSE,
  quiet = FALSE
)
```

Arguments

paths	'Charachter' or 'character vector', path specifying the location of the 'eq_classes.txt' files generated with salmon.
tx2gene	A 'dataframe' linking transcript identifiers to their corresponding gene identifiers. Transcript identifiers must be in a column 'isoform_id'. Corresponding gene identifiers must be in a column 'gene_id'.
multigene	'Logical', should equivalence classes that are compatible with multiple genes be retained? Default is 'FALSE', removing such ambiguous equivalence classes.
ignoreTxVersion	logical, whether to split the isoform id on the '.' character to remove version information to facilitate matching with the isoform id in 'tx2gene' (default FALSE).
ignoreAfterBar	logical, whether to split the isoform id on the '|' character to facilitate matching with the isoform id in 'tx2gene' (default FALSE).
quiet	'Logical', set 'TRUE' to avoid displaying messages.

Value

A list with two elements. The first element 'counts' is a sparse count matrix with equivalence class identifiers in the rows. If multiple paths are specified, the columns are in the same order as the paths. The second element 'tx2gene_matched' allows for linking those identifiers to their respective transcripts and genes.

Details

The resulting count matrix uses equivalence class identifiers as rownames. These can be linked to respective transcripts and genes using the 'tx2gene_matched' element of the output. Specifically, if the equivalence class identifier reads 1|2|8, then the equivalence class is compatible with the transcripts and their respective genes in rows 1, 2 and 8 of 'tx2gene_matched'.

Author(s)

Jeroen Gilis

`scaleInfReps` *Scale inferential replicate counts*

Description

A helper function to scale the inferential replicates to the mean sequencing depth. The scaling takes into account a robust estimator of size factor (median ratio method is used). First, counts are corrected per row using the effective lengths (for gene counts, the average transcript lengths), then scaled per column to the geometric mean sequence depth, and finally are adjusted per-column up or down by the median ratio size factor to minimize systematic differences across samples.

Usage

```
scaleInfReps(
  y,
  lengthCorrect = TRUE,
  meanDepth = NULL,
  sfFun = NULL,
  minCount = 10,
  minN = 3,
  saveMeanScaled = FALSE,
  quiet = FALSE
)
```

Arguments

`y`	a SummarizedExperiment with: `infReps` a list of inferential replicate count matrices, `counts` the estimated counts matrix, and `length` the effective lengths matrix
`lengthCorrect`	whether to use effective length correction (default is TRUE)
`meanDepth`	(optional) user can specify a different mean sequencing depth. By default the geometric mean sequencing depth is computed
`sfFun`	(optional) size factors function. An alternative to the median ratio can be provided here to adjust the scaledTPM so as to remove remaining library size differences. Alternatively, one can provide a numeric vector of size factors
`minCount`	for internal filtering, the minimum count

minN	for internal filtering, the minimum sample size at minCount
saveMeanScaled	store the mean of scaled inferential replicates as an assay 'meanScaled'
quiet	display no messages

Value

a SummarizedExperiment with the inferential replicates as scaledTPM with library size already corrected (no need for further normalization). A column log10mean is also added which is the log10 of the mean of scaled counts across all samples and all inferential replicates.

Examples

```
y <- makeSimSwishData()
y <- scaleInfReps(y)
```

---

splitSwish *Function for splitting SummarizedExperiment into separate RDS files*

Description

The splitSwish function splits up the y object along genes and writes a Snakefile that can be used with Snakemake to distribute running swish across genes. This workflow is primarily designed for large single cell datasets, and so the default is to not perform length correction within the distributed jobs. See the alevin section of the vignette for an example. See the Snakemake documention for details on how to run and customize a Snakefile: https://snakemake.readthedocs.io

Usage

```
splitSwish(y, nsplits, prefix = "swish", snakefile = NULL, overwrite = FALSE)
```

Arguments

y	a SummarizedExperiment
nsplits	integer, how many pieces to break y into
prefix	character, the path of the RDS files to write out, e.g. prefix="/path/to/swish" will generate swish.rds files at this path
snakefile	character, the path of a Snakemake file, e.g. Snakefile, that should be written out. If NULL, then no Snakefile is written out
overwrite	logical, whether the snakefile and RDS files (swish1.rds, ...) should overwrite existing files

Value

nothing, files are written out

References

Compression and splitting across jobs:

Van Buren, S., Sarkar, H., Srivastava, A., Rashid, N.U., Patro, R., Love, M.I. (2020) Compression of quantification uncertainty for scRNA-seq counts. bioRxiv. https://doi.org/10.1101/2020.07.06.189639

Snakemake:

Koster, J., Rahmann, S. (2012) Snakemake - a scalable bioinformatics workflow engine. Bioinformatics. https://doi.org/10.1093/bioinformatics/bts480

`swish` *Swish method: differential expression accounting for inferential uncertainty*

Description

The Swish method, or "SAMseq With Inferential Samples Helps". Performs non-parametric inference on rows of `y` for various experimental designs. See References for details.

Usage

```
swish(
  y,
  x,
  cov = NULL,
  pair = NULL,
  interaction = FALSE,
  cor = c("none", "spearman", "pearson"),
  nperms = 100,
  estPi0 = FALSE,
  qvaluePkg = "qvalue",
  pc = 5,
  nRandomPairs = 30,
  fast = NULL,
  returnNulls = FALSE,
  quiet = FALSE
)
```

Arguments

`y`	a SummarizedExperiment containing the inferential replicate matrices of median-ratio-scaled TPM as assays 'infRep1', 'infRep2', etc.
`x`	the name of the condition variable. A factor with two levels for a two group analysis (possible to adjust for covariate or matched samples, see next two arguments). The log fold change is computed as non-reference level over reference level (see vignette: 'Note on factor levels')

cov	the name of the covariate for adjustment. If provided a stratified Wilcoxon in performed. Cannot be used with pair, unless using either interaction or cor
pair	the name of the pair variable, which should be the number of the pair. Can be an integer or factor. If specified, a signed rank test is used to build the statistic by default. **Note:** For simple paired designs, see use of fast=1 for a much faster implementation of paired testing using a one-sample z-score test statistic. All samples across x must be pairs if this argument is specified. Cannot be used with cov, unless using either interaction or cor
interaction	logical, whether to perform a test of an interaction between x and cov. Can use pair or not. See Details.
cor	character, whether to compute correlation of x with the log counts, and signifance testing on the correlation as a test statistic. Either "spearman" or "pearson" correlations can be computed. For Spearman the correlation is computed over ranks of x and ranks of inferential replicates. For Pearson, the correlation is computed for x and log2 of the inferential replicates plus pc. Default is "none", e.g. two-group comparison using the rank sum test or other alternatives listed above. Additionally, correlation can be computed between a continuous variable cov and log fold changes across x matched by pair
nperms	the number of permutations. if set above the possible number of permutations, the function will print a message that the value is set to the maximum number of permutations possible
estPi0	logical, whether to estimate pi0
qvaluePkg	character, which package to use for q-value estimation, samr or qvalue
pc	pseudocount for finite estimation of log2FC, not used in calculation of test statistics, locfdr or qvalue
nRandomPairs	the number of random pseudo-pairs (only used with interaction=TRUE and un-matched samples) to use to calculate the test statistic
fast	an integer (0 or 1), toggles different methods based on speed, currently only relevant for simple paired analysis. For simple paired design, fast=1 triggers the use of a one-sample z-score instead of a signed rank statistic. The one-sample z-score is much faster (can be >10x faster), by avoiding the expensive re-computation of ranks during permutations. fast=1 is not relevant for interaction or cor type designs
returnNulls	logical, only return the stat vector, the log2FC vector, and the nulls matrix (default FALSE)
quiet	display no messages

Details

interaction: The interaction tests are different than the other tests produced by swish, in that they focus on a difference in the log2 fold change across levels of x when comparing the two levels in cov. If pair is specified, this will perform a Wilcoxon rank sum test on the two groups of matched sample LFCs. If pair is not included, multiple random pairs of samples within the two groups are chosen, and again a Wilcoxon rank sum test compared the LFCs across groups.

Value

a SummarizedExperiment with metadata columns added: the statistic (either a centered Wilcoxon Mann-Whitney or a signed rank statistic, aggregated over inferential replicates), a log2 fold change (the median over inferential replicates, and averaged over pairs or groups (if groups, weighted by sample size), the local FDR and q-value, as estimated by the samr package.

References

The citation for swish method is:

Anqi Zhu, Avi Srivastava, Joseph G Ibrahim, Rob Patro, Michael I Love "Nonparametric expression analysis using inferential replicate counts" Nucleic Acids Research (2019). https://doi.org/10.1093/nar/gkz622

The swish method builds upon the SAMseq method, and extends it by incorporating inferential uncertainty, as well as providing methods for additional experimental designs (see vignette).

For reference, the publication describing the SAMseq method is:

Jun Li and Robert Tibshirani "Finding consistent patterns: A nonparametric approach for identifying differential expression in RNA-Seq data" Stat Methods Med Res (2013). https://doi.org/10.1177/0962280211428386

Examples

```
library(SummarizedExperiment)
set.seed(1)
y <- makeSimSwishData()
y <- scaleInfReps(y)
y <- labelKeep(y)
y <- swish(y, x="condition")

# histogram of the swish statistics
hist(mcols(y)$stat, breaks=40, col="grey")
cols = rep(c("blue","purple","red"),each=2)
for (i in 1:6) {
  arrows(mcols(y)$stat[i], 20,
         mcols(y)$stat[i], 10,
         col=cols[i], length=.1, lwd=2)
}

# plot inferential replicates
plotInfReps(y, 1, "condition")
plotInfReps(y, 3, "condition")
plotInfReps(y, 5, "condition")
```

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