

# Package ‘fishpond’

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**Title** Fishpond: differential transcript and gene expression with inferential replicates

**Version** 2.1.0

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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 utilities for working with Salmon and Alevin quantification files.

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

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fishpond-package      *Downstream methods for Salmon and Alevin expression data*

---

### Description

This package provides statistical methods and other tools for working with Salmon and Alevin quantification of RNA-seq data. In particular, it contains the Swish non-parametric method for detecting differential transcript expression (DTE). Swish can also be used to detect differential gene expression (DGE).

## Details

The main 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](#)
- [isoformProportions](#) - convert counts to isoform proportions
- [makeInfReps](#) - create pseudo-inferential replicates
- [splitSwish](#) - split Swish analysis across jobs with Snakemake

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>

## Author(s)

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## 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>

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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 <a href="#">swish</a>

**Value**

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

---

computeInfrRV	<i>Compute inferential relative variance (InfrRV)</i>
---------------	---

---

**Description**

InfrRV is used the Swish publication for visualization. This function provides computation of the mean InfrRV, a simple statistic that measures inferential uncertainty. It also computes and adds the mean and variance of inferential replicates, which can be useful ahead of [plotInfReps](#). Note that InfrRV is not used in the swish statistical method at all, it is just for visualization. See function code for details.

**Usage**

```
computeInfrRV(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

**Value**

a SummarizedExperiment with meanInfrRV in the metadata columns

---

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

---

## Description

The DESeq2-apeglm With Inferential Samples implementation supposes a hierarchical distribution of log<sub>2</sub> 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

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

## Value

a SummarizedExperiment with metadata columns added: the log<sub>2</sub> fold change and posterior SD using inferential replicates, and the original log<sub>2</sub> 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	<i>Obtain a trace of inferential replicates for a sample</i>
----------	--

---

### 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

<code>y</code>	a SummarizedExperiment with inferential replicates as assays <code>infRep1</code> etc.
<code>idx</code>	the names or row numbers of the gene or transcript to plot
<code>samp_idx</code>	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	<i>Import allelic counts as a SummarizedExperiment</i>
---------------------	--

---

### 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` (`a1`) and `ENST123_ref` (`a2`). There must be exactly two alleles for each reference transcript, and the `--keep-duplicates` option should be used in Salmon indexing to avoid removing 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 alternative allele, and `a2` is used as the reference allele (and therefore "`a2`" is the reference level of the `allele` factor that is returned in the `colData`).

**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/alternative allele
a2	the symbol for the reference 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 reference allele (a2) comes first, then the alternative allele (a1), e.g. [a2   a1]. 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). Either this should include the a1 and a2 suffix for the transcripts and genes, 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 not provided, the output object will be transcript-level. Note: do not attempt to set the txOut argument, it will conflict with internal calls to downstream functions. Note: 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.
...	any arguments to pass to tximeta

**Details**

Requires the tximeta package. 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 software.

**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", and so comparisons by default will be a1/a2 (alt/ref).

---

isoformProportions	<i>Create isoform proportions from scaled data</i>
--------------------	--

---

### 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

<code>y</code>	a SummarizedExperiment
<code>geneCol</code>	the name of the gene ID column in the metadata columns for the rows of <code>y</code>
<code>quiet</code>	display no messages

### Value

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

---

labelKeep	<i>Label rows to keep based on minimal count</i>
-----------	--

---

### 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

<code>y</code>	a SummarizedExperiment
<code>minCount</code>	the minimum count
<code>minN</code>	the minimum sample size at <code>minCount</code>
<code>x</code>	the name of the condition variable, will use the smaller of the two groups to set <code>minN</code> . Similar to edgeR's <code>filterByExpr</code> , as the smaller group grows past 10, <code>minN</code> 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. Alevin-fry - <https://www.biorxiv.org/content/10.1101/2021.06.29.450377v1>

**Usage**

```
load_fry_raw(fryDir, quiet = FALSE)
```

```
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
quiet	logical whether to display no messages
outputFormat	can be <i>either</i> be a list that defines the desired format of the output SingleCellExperiment object <i>or</i> a string that represents one of the pre-defined output formats, which are "scRNA", "snRNA", "scVelo" and "velocity". 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.

**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.

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

**"raw"**: This format put 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.

### Details about load\_fry\_raw

This function processes `alevin-fry`'s quantification result contained within the input folder. This function returns a list that consists of the gene count matrix, the gene names list, the barcode list, and some metadata, such as the number of genes in the experiment and whether `alevin-fry` was executed in USA mode. In the returned list, the all-in-one count matrix, `count_mat`, returned from the USA mode of `alevin-fry` consists of the spliced count of genes defined in `gene.names` for all barcodes defined in `barcodes`, followed by the unspliced count of genes in the same order for all cells, then followed by the ambiguous count of genes in the same order for all cells.

**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 avelin-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
)
```

## 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

## Value

a SummarizedExperiment

## Examples

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

---

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

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

## 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

---

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
)
```

## 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 $qnorm(.975) \approx 1.96$ corresponding to 95 intervals
applySF	logical, when inferential replicates are not present, should <code>y\$sizeFactor</code> be divided out from the mean and interval plots (default FALSE)
reorder	logical, should points within a group defined by condition and covariate be re-ordered 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)

**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	<i>MA plot</i>
-------------	----------------

---

**Description**

MA plot

**Usage**

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

**Arguments**

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

**Value**

nothing, a plot is displayed

**Examples**

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

---

readEDS	<i>readEDS - a utility function for quickly reading in Alevin's EDS format</i>
---------	--

---

**Description**

readEDS - a utility function for quickly reading in Alevin's EDS format

**Usage**

```
readEDS(numOfGenes, numOfOriginalCells, countMatFilename, tierImport = FALSE)
```



**Arguments**

numOfGenes        number of genes  
 numOfOriginalCells        number of cells  
 countMatFilename        pointer to the EDS file, quants\_mat.gz  
 tierImport        whether the countMatFilename refers to a quants tier file

**Value**

a genes x cells sparse matrix, of the class dgMatrix

---

scaleInfReps	<i>Scale inferential replicate counts</i>
--------------	---

---

**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	<i>Function for splitting SummarizedExperiment into separate RDS files</i>
------------	--

---

**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 documentation 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: SAMseq With Inferential Samples Helps*

---

**Description**

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 = 1,  
  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 is performed. Cannot be used with pair (unless using 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. All samples across x must be pairs if this is specified. Cannot be used with cov (unless using cor)
interaction	logical, whether to perform a test of an interaction between x and cov. See Details.
cor	character, whether to compute correlation of x with the log counts, and significance 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, toggles different methods based on speed (fast=1 is default, 0 is slower). See Details.
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.

**fast:** '0' involves recomputing ranks of the inferential replicates for each permutation, '1' (default) is roughly 10x faster by avoiding re-computing ranks for each permutation. The fast argument is only relevant for the following three experimental designs: (1) two group Wilcoxon, (2) stratified

Wilcoxon, e.g. cov is specified, and (3) the paired interaction test, e.g. pair and cov are specified. For paired design and general interaction test, there are not fast/slow alternatives.

### 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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