Package 'tximport'

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Title Import and summarize transcript-level estimates for transcriptand gene-level analysis

Description Imports transcript-level abundance, estimated counts and transcript lengths, and summarizes into matrices for use with downstream gene-level analysis packages. Average transcript length, weighted by sample-specific transcript abundance estimates, is provided as a matrix which can be used as an offset for different expression of gene-level counts.

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VignetteBuilder knitr

Imports utils, stats, methods

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URL https://github.com/mikelove/tximport

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makeCountsFromAbundance

Low-level function to make counts from abundance using matrices

Description

Simple low-level function used within tximport to generate scaledTPM or lengthScaledTPM counts, taking as input the original counts, abundance and length matrices. NOTE: This is a low-level function exported in case it is needed for some reason, but the recommended way to generate counts-from-abundance is using tximport with the countsFromAbundance argument.

Usage

```
makeCountsFromAbundance(countsMat, abundanceMat, lengthMat,
  countsFromAbundance = c("scaledTPM", "lengthScaledTPM"))
```

Arguments

countsMat a matrix of original counts

abundanceMat a matrix of abundances (typically TPM)

lengthMat a matrix of effective lengths

 $\verb|countsFromAbundance||$

the desired type of count-from-abundance output

Value

a matrix of count-scale data generated from abundances. for details on the calculation see tximport.

summarizeToGene

Summarize estimated quantitites to gene-level

Description

Summarizes abundances, counts, lengths, (and inferential replicates or variance) from transcript- to gene-level.

Usage

```
summarizeToGene(object, ...)

## S4 method for signature 'list'
summarizeToGene(object, tx2gene, varReduce = FALSE,
  ignoreTxVersion = FALSE, ignoreAfterBar = FALSE,
  countsFromAbundance = c("no", "scaledTPM", "lengthScaledTPM"))
```

Arguments

object the list of matrices of trancript-level abundances, counts, lengths produced by

tximport, with a countsFromAbundance element that tells how the counts were

generated.

... additional arguments, ignored

tx2gene see tximport
varReduce see tximport
ignoreTxVersion

see tximport

ignoreAfterBar see tximport
countsFromAbundance

see tximport

Value

a list of matrices of gene-level abundances, counts, lengths, (and inferential replicates or variance if inferential replicates are present).

See Also

tximport

tximport Import transcript-level abundances and counts for transcript- and gene-level analysis packages

Description

tximport imports transcript-level estimates from various external software and optionally summarizes abundances, counts, and transcript lengths to the gene-level (default) or outputs transcript-level matrices (see tx0ut argument).

Usage

```
tximport(files, type = c("none", "salmon", "sailfish", "alevin",
   "kallisto", "rsem", "stringtie"), txIn = TRUE, txOut = FALSE,
   countsFromAbundance = c("no", "scaledTPM", "lengthScaledTPM",
   "dtuScaledTPM"), tx2gene = NULL, varReduce = FALSE,
   dropInfReps = FALSE, infRepStat = NULL, ignoreTxVersion = FALSE,
   ignoreAfterBar = FALSE, geneIdCol, txIdCol, abundanceCol, countsCol,
   lengthCol, importer = NULL, existenceOptional = FALSE,
   sparse = FALSE, sparseThreshold = 1, readLength = 75)
```

Arguments

files a character vector of filenames for the transcript-level abundances

type character, the type of software used to generate the abundances. Options are "salmon", "sailfish", "alevin", "kallisto", "rsem", "stringtie", or "none". This

argument is used to autofill the arguments below (geneIdCol, etc.) "none" means

that the user will specify these columns.

txIn logical, whether the incoming files are transcript level (default TRUE)

txOut logical, whether the function should just output transcript-level (default FALSE)

countsFromAbundance

character, either "no" (default), "scaledTPM", "lengthScaledTPM", or "dtuScaledTPM". Whether to generate estimated counts using abundance estimates:

• scaled up to library size (scaledTPM),

- scaled using the average transcript length over samples and then the library size (lengthScaledTPM), or
- scaled using the median transcript length among isoforms of a gene, and then the library size (dtuScaledTPM).

dtuScaledTPM is designed for DTU analysis in combination with txOut=TRUE, and it requires specifing a tx2gene data.frame. dtuScaledTPM works such that within a gene, values from all samples and all transcripts get scaled by the same fixed median transcript length. If using scaledTPM, lengthScaledTPM, or gene-LengthScaledTPM, the counts are no longer correlated across samples with transcript length, and so the length offset matrix should not be used.

tx2gene a two-column data.frame linking transcript id (column 1) to gene id (column 2).

the column names are not relevant, but this column order must be used. this argument is required for gene-level summarization for methods that provides

transcript-level estimates only (kallisto, Salmon, Sailfish)

varReduce whether to reduce per-sample inferential replicates information into a matrix of

sample variances variance (default FALSE)

dropInfReps whether to skip reading in inferential replicates (default FALSE)

infRepStat a function to re-compute counts and abundances from the inferential replicates,

e.g. matrixStats::rowMedians to re-compute counts as the median of the inferential replicates. The order of operations is: first counts are re-computed, then abundances are re-computed. Following this, if countsFromAbundance is not "no", tximport will again re-compute counts from the re-computed abundances. infRepStat should operate on rows of a matrix. (default is NULL)

ignoreTxVersion

logical, whether to split the tx id on the '.' character to remove version informa-

tion, for easier matching with the tx id in gene2tx (default FALSE)

ignoreAfterBar logical, whether to split the tx id on the 'l' character (default FALSE)

geneIdCol name of column with gene id. if missing, the gene2tx argument can be used

txIdCol name of column with tx id

abundanceCol name of column with abundances (e.g. TPM or FPKM)

countsCol name of column with estimated counts

lengthCol name of column with feature length information

importer a function used to read in the files

existenceOptional

logical, should tximport not check if files exist before attempting import (default

FALSE, meaning files must exist according to file.exists)

sparse logical, whether to try to import data sparsely (default is FALSE). Initial imple-

mentation for txOut=TRUE, countsFromAbundance="no" or "scaledTPM", no inferential replicates. Only counts matrix is returned (and abundance matrix if

using "scaledTPM")

sparseThreshold

the minimum threshold for including a count as a non-zero count during sparse

import (default is 1)

readLength numeric, the read length used to calculate counts from StringTie's output of

coverage. Default value (from StringTie) is 75. The formula used to calculate

counts is: cov * transcript length / read length

Details

tximport will also load in information about inferential replicates – a list of matrices of the Gibbs samples from the posterior, or bootstrap replicates, per sample – if these data are available in the expected locations relative to the files. The inferential replicates, stored in infReps in the output list, are on estimated counts, and therefore follow counts in the output list. By setting varReduce=TRUE, the inferential replicate matrices will be replaced by a single matrix with the sample variance per transcript/gene and per sample.

While tximport summarizes to the gene-level by default, the user can also perform the import and summarization steps manually, by specifing txOut=TRUE and then using the function summarizeToGene. Note however that this is equivalent to tximport with txOut=FALSE (the default).

Solutions to the error "tximport failed at summarizing to the gene-level":

- 1. provide a tx2gene data.frame linking transcripts to genes (more below)
- 2. avoid gene-level summarization by specifying txOut=TRUE
- 3. set geneIdCol to an appropriate column in the files

See vignette('tximport') for example code for generating a tx2gene data.frame from a TxDb object. Note that the keys and select functions used to create the tx2gene object are documented in the man page for AnnotationDb-class objects in the AnnotationDbi package (TxDb inherits from AnnotationDb). For further details on generating TxDb objects from various inputs see vignette('GenomicFeatures') from the GenomicFeatures package.

For type="alevin" all arguments other than files are ignored, and files should point to a single quants_mat.gz file, in the directory structure created by the alevin software (e.g. do not move the file or delete the other important files). tximport is solely importing the gene-by-cell matrix of counts, as txi\$counts, and effective lengths are not estimated. Length correction should not be applied to datasets where there is not an expected correlation of counts and feature length.

Value

a simple list containing matrices: abundance, counts, length. Another list element 'countsFromAbundance' carries through the character argument used in the tximport call. If detected, and txOut=TRUE, inferential replicates for each sample will be imported and stored as a list of matrices, itself an element infReps in the returned list. If varReduce=TRUE the inferential replicates will be summarized according to the sample variance, and stored as a matrix variance. The length matrix contains the average transcript length for each gene which can be used as an offset for gene-level analysis.

References

Charlotte Soneson, Michael I. Love, Mark D. Robinson (2015): Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Research. http://dx.doi.org/10.12688/f1000research.7563.1

Examples

```
# load data for demonstrating tximport
# note that the vignette shows more examples
# including how to read in files quickly using the readr package
library(tximportData)
dir <- system.file("extdata", package="tximportData")
samples <- read.table(file.path(dir, "samples.txt"), header=TRUE)
files <- file.path(dir, "salmon", samples$run, "quant.sf.gz")
names(files) <- paste0("sample",1:6)

# tx2gene links transcript IDs to gene IDs for summarization
tx2gene <- read.csv(file.path(dir, "tx2gene.gencode.v27.csv"))
txi <- tximport(files, type="salmon", tx2gene=tx2gene)</pre>
```

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