

Package ‘seeker’

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Type Package

Title Simplified Fetching and Processing of Microarray and RNA-Seq Data

Version 1.0.9

Description Wrapper around various existing tools and command-line interfaces, providing a standard interface, simple parallelization, and detailed logging. For microarray data, maps probe sets to standard gene IDs, building on 'GEOquery' Davis and Meltzer (2007) <doi:10.1093/bioinformatics/btm254>, 'ArrayExpress' Kauffmann et al. (2009) <doi:10.1093/bioinformatics/btp354>, Robust multi-array average 'RMA' Irizarry et al. (2003) <doi:10.1093/biostatistics/4.2.249>, and 'BrainArray' Dai et al. (2005) <doi:10.1093/nar/gni179>. For RNA-seq data, fetches metadata and raw reads from National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA), performs standard adapter and quality trimming using 'TrimGalore' Krueger <<https://github.com/FelixKrueger/TrimGalore>>, performs quality control checks using 'FastQC' Andrews <<https://github.com/s-andrews/FastQC>>, quantifies transcript abundances using 'salmon' Patro et al. (2017) <doi:10.1038/nmeth.4197> and potentially 'refgenie' Stolarczyk et al. (2020) <doi:10.1093/gigascience/giz149>, aggregates the results using 'MultiQC' Ewels et al. (2016) <doi:10.1093/bioinformatics/btw354>, maps transcripts to genes using 'biomaRt' Durinkck et al. (2009) <doi:10.1038/nprot.2009.97>, and summarizes transcript-level quantifications for gene-level analyses using 'tximport' Soneson et al. (2015) <doi:10.12688/f1000research.7563.2>.

URL <https://seeker.hugheylib.org>, <https://github.com/hugheylib/seeker>

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R topics documented:

checkDefaultCommands	2
fastqc	3
fastqscreen	4
fetch	4
fetchMetadata	6
getPlatforms	6
getSalmonMetadata	7
getTx2gene	7
installCustomCdfPackages	8
installSysDeps	9
multiqc	10
salmon	10
seeker	11
seekerArray	14
trimgalore	16
tximport	17
Index	18

checkDefaultCommands *Check for presence of command-line interfaces*

Description

This function checks whether the command-line tools used by seeker are accessible in the expected places.

Usage

```
checkDefaultCommands(keepIdx = FALSE)
```

Arguments

keepIdx Logical indicating whether or not to keep the idx column of d.

Value

A data.table with columns for command, path, and version.

fastqc	<i>Run FastQC</i>
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Description

This function calls `fastqc` using `system2()`. To run in parallel, register a parallel backend, e.g., using `doParallel::registerDoParallel()`.

Usage

```
fastqc(filepaths, outputDir = "fastqc_output", cmd = "fastqc", args = NULL)
```

Arguments

filepaths Paths to fastq files. For single-end reads, each element should be a single filepath. For paired-end reads, each element can be two filepaths separated by ";".

outputDir Directory in which to store output. Will be created if it doesn't exist.

cmd Name or path of the command-line interface.

args Additional arguments to pass to the command-line interface.

Value

A vector of exit codes, invisibly.

fastqscreen	<i>Run FastQ Screen</i>
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Description

This function calls `fastq_screen` using `system2()`. To run in parallel, register a parallel backend, e.g., using `doParallel::registerDoParallel()`.

Usage

```
fastqscreen(
  filepaths,
  outputDir = "fastqscreen_output",
  cmd = "fastq_screen",
  args = c("--threads", foreach::getDoParWorkers(), "--conf",
           "~/FastQ_Screen_Genomes/fastq_screen.conf")
)
```

Arguments

filepaths	Paths to fastq files. For single-end reads, each element should be a single filepath. For paired-end reads, each element can be two filepaths separated by ";".
outputDir	Directory in which to store output. Will be created if it doesn't exist.
cmd	Name or path of the command-line interface.
args	Additional arguments to pass to the command-line interface.

Value

A vector of exit codes, invisibly.

fetch	<i>Fetch files</i>
-------	--------------------

Description

This function uses the NCBI SRA Toolkit via `system2()` to download files from SRA and convert them to fastq.gz. To process files in parallel, register a parallel backend, e.g., using `doParallel::registerDoParallel()`. Beware that intermediate files created by `fasterq-dump` are uncompressed and could require hundreds of gigabytes if files are processed in parallel.

Usage

```
fetch(  
  accessions,  
  outputDir,  
  overwrite = FALSE,  
  keepSra = FALSE,  
  prefetchCmd = "prefetch",  
  prefetchArgs = NULL,  
  fasterqdumpCmd = "fasterq-dump",  
  fasterqdumpArgs = NULL,  
  pigzCmd = "pigz",  
  pigzArgs = NULL  
)
```

Arguments

accessions	Character vector of SRA run accessions.
outputDir	String indicating the local directory in which to save the files. Will be created if it doesn't exist.
overwrite	Logical indicating whether to overwrite files that already exist in outputDir.
keepSra	Logical indicating whether to keep the ".sra" files.
prefetchCmd	String indicating command for prefetch, which downloads ".sra" files.
prefetchArgs	Character vector indicating arguments to pass to prefetch.
fasterqdumpCmd	String indicating command for fasterq-dump, which uses ".sra" files to create ".fastq" files.
fasterqdumpArgs	Character vector indicating arguments to pass to fasterq-dump.
pigzCmd	String indicating command for pigz, which converts ".fastq" files to ".fastq.gz" files.
pigzArgs	Character vector indicating arguments to pass to pigz.

Value

A list. As the function runs, it updates a tab-delimited log file in outputDir called "progress.tsv".

See Also

[fetchMetadata\(\)](#)

fetchMetadata	<i>Fetch metadata for a genomic study</i>
---------------	---

Description

This function can use the API of the European Nucleotide Archive (recommended) or the Sequence Read Archive.

Usage

```
fetchMetadata(  
  bioproject,  
  host = c("ena", "sra"),  
  fields = c("study_accession", "sample_accession", "secondary_sample_accession",  
            "sample_alias", "sample_title", "experiment_accession", "run_accession", "fastq_md5",  
            "fastq_ftp", "fastq_aspera")  
)
```

Arguments

bioproject	String indicating bioproject accession.
host	String indicating from where to fetch the metadata.
fields	Character vector indicating which fields to fetch, if host is "ena".

Value

A data.table.

See Also

[fetch\(\)](#)

getPlatforms	<i>Get supported microarray platforms</i>
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Description

Get supported microarray platforms

Usage

```
getPlatforms(type = c("cdf", "mapping"))
```

Arguments

type String indicating whether to get supported platforms for processing raw Affymetrix data using custom CDF or for mapping already processed data from probes to genes.

Value

A data.table.

getSalmonMetadata *Aggregate metadata from salmon quantifications*

Description

Aggregate metadata from salmon quantifications

Usage

```
getSalmonMetadata(inputDir, outputDir = "data")
```

Arguments

inputDir Directory that contains output from salmon.
outputDir Directory in which to save the result, a file named "salmon_meta_info.csv". If NULL, no file is saved.

Value

A data.table, invisibly.

getTx2gene *Get mapping between transcripts and genes*

Description

This function uses the [biomaRt package](#).

Usage

```
getTx2gene(  
  organism = "mmusculus",  
  version = NULL,  
  outputDir = "data",  
  checkArgsOnly = FALSE  
)
```

Arguments

organism	String used to pass <code>paste0(organism, "_gene_ensembl")</code> as the dataset argument to <code>biomaRt::useEnsembl()</code> . To see available datasets, do <code>mart = biomaRt::useEnsembl("gene")</code> .
version	Passed to <code>biomaRt::useEnsembl()</code> . NULL indicates the latest version. To see available versions, do <code>biomaRt::listEnsemblArchives()</code> .
outputDir	Directory in which to save the result, a file named "tx2gene.csv.gz". If NULL, no file is saved.
checkArgsOnly	Logical indicating whether to only check function arguments. Used for testing.

Value

If `checkArgsOnly` is FALSE, a `data.table` based on the result from `biomaRt::getBM()`, with an attribute "version". Otherwise `0`.

installCustomCdfPackages

Install custom CDF packages

Description

Install Brainarray custom CDFs for processing raw Affymetrix data. See http://brainarray.mbi.med.umich.edu/Brainarray/Database/CustomCDF/CDF_download.asp.

Usage

```
installCustomCdfPackages(pkgs, ver = 25, dryRun = FALSE)
```

Arguments

pkgs	Character vector of package names, e.g., "hgu133ahsentrezgcdf".
ver	Integer version number (25 as of 5 Jan 2021).
dryRun	Logical indicating whether to actually install the packages.

Value

A character vector of URLs, invisibly.

installSysDeps	<i>Install seeker's system dependencies</i>
----------------	---

Description

This function installs and configures the various programs required for seeker to fetch and process RNA-seq data.

Usage

```
installSysDeps(  
  sraToolkitDir,  
  minicondaDir,  
  refgenieDir,  
  rprofileDir,  
  minicondaEnv = "seeker",  
  refgenieGenomes = NULL,  
  fastqscreenDir = NULL  
)
```

Arguments

sraToolkitDir	String indicating directory in which to install the SRA Toolkit . Recommended to use "~", the home directory. If NULL, the Toolkit will not be installed.
minicondaDir	String indicating directory in which to install Miniconda . Recommended to use "~", the home directory. If NULL, Miniconda will not be installed.
refgenieDir	String indicating directory in which to store the directory of genome assets from refgenie, which will be named "refgenie_genomes". Recommended to use "~", the home directory. Only used if minicondaDir is not NULL.
rprofileDir	String indicating directory in which to create or modify .Rprofile, which is run by R on startup. Common options are "~" or ".".
minicondaEnv	String indicating name of the Miniconda environment in which to install various conda packages (fastq-screen, fastqc, multiqc, pigz, refgenie, salmon, and trim-galore).
refgenieGenomes	Character vector indicating genome assets, such as transcriptome indexes for salmon() , to pull from refgenomes using refgenie. If NULL, no assets are fetched.
fastqscreenDir	String indicating directory in which to download the genomes for fastqscreen() . This takes a long time. If NULL, genomes are not downloaded.

Value

NULL, invisibly

See Also

[seeker\(\)](#)

multiqc	<i>Run MultiQC</i>
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Description

This function calls `multiqc` using `system2()`.

Usage

```
multiqc(  
  parentDir = ".",  
  outputDir = "multiqc_output",  
  cmd = "multiqc",  
  args = NULL  
)
```

Arguments

<code>parentDir</code>	Directory that contains output to be aggregated.
<code>outputDir</code>	Directory in which to store output. Will be created if it doesn't exist.
<code>cmd</code>	Name or path of the command-line interface.
<code>args</code>	Additional arguments to pass to the command-line interface.

Value

An exit code, invisibly.

salmon	<i>Run Salmon</i>
--------	-------------------

Description

This function calls `salmon` using `system2()`. To run in parallel, register a parallel backend, e.g., using `doParallel::registerDoParallel()`.

Usage

```
salmon(  
  filepaths,  
  samples,  
  indexDir,  
  outputDir = "salmon_output",  
  cmd = "salmon",  
  args = c("-l A -q --seqBias --gcBias --no-version-check -p",  
    foreach::getDoParWorkers()),  
  compress = TRUE  
)
```

Arguments

filepaths	Paths to fastq files. For single-end reads, each element should be a single filepath. For paired-end reads, each element should be two filepaths separated by ";".
samples	Corresponding sample names for fastq files.
indexDir	Directory that contains salmon index.
outputDir	Directory in which to store output. Will be created if it doesn't exist.
cmd	Name or path of the command-line interface.
args	Additional arguments to pass to the command-line interface.
compress	Logical indicating whether to gzip the quantification file (quant.sf) from salmon. Does not affect downstream analysis.

Value

A vector of exit codes, invisibly.

seeker	<i>Process RNA-seq data end to end</i>
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Description

This function selectively performs various steps to process RNA-seq data.

Usage

```
seeker(params, parentDir = ".", dryRun = FALSE)
```

Arguments

params	Named list of parameters with components: <ul style="list-style-type: none"> • study: String used to name the output directory within parentDir. • metadata: Named list with components: <ul style="list-style-type: none"> – run: Logical indicating whether to fetch metadata. See fetchMetadata(). If TRUE, saves a file parentDir/study/metadata.csv. If FALSE, expects that file to already exist. The unmodified fetched or found metadata is saved to a file parentDir/study/metadata_original.csv. Following components are only checked if run is TRUE. – bioproject: String indicating the study's bioproject accession. – include: Optional named list for specifying which rows of metadata to include for further processing, with components: <ul style="list-style-type: none"> * colname: String indicating column in metadata * values: Vector indicating values within colname
--------	---

- exclude: Optional named list for specifying which rows of metadata to exclude from further processing (superseding include), with components:
 - * colname: String indicating column in metadata
 - * values: Vector indicating values within colname
- fetch: Named list with components:
 - run: Logical indicating whether to fetch files from SRA. See [fetch\(\)](#). If TRUE, saves files to `parentDir/study/fetch_output`. Whether TRUE or FALSE, expects metadata to have a column "run_accession", and updates metadata with column "fastq_fetched" containing paths to files in `parentDir/study/fetch_output`. Following components are only checked if run is TRUE.
 - keep: Logical indicating whether to keep fastq.gz files when all processing steps have completed. NULL indicates TRUE.
 - overwrite: Logical indicating whether to overwrite files that already exist. NULL indicates to use the default in [fetch\(\)](#).
 - keepSra: Logical indicating whether to keep the ".sra" files. NULL indicates to use the default in [fetch\(\)](#).
 - prefetchCmd: String indicating command for prefetch, which downloads ".sra" files. NULL indicates to use the default in [fetch\(\)](#).
 - prefetchArgs: Character vector indicating arguments to pass to prefetch. NULL indicates to use the default in [fetch\(\)](#).
 - fasterqdumpCmd: String indicating command for fasterq-dump, which uses ".sra" files to create ".fastq" files. NULL indicates to use the default in [fetch\(\)](#).
 - prefetchArgs: Character vector indicating arguments to pass to fasterq-dump. NULL indicates to use the default in [fetch\(\)](#).
 - pigzCmd: String indicating command for pigz, which converts ".fastq" files to ".fastq.gz" files. NULL indicates to use the default in [fetch\(\)](#).
 - pigzArgs: Character vector indicating arguments to pass to pigz. NULL indicates to use the default in [fetch\(\)](#).
- trimgalore: Named list with components:
 - run: Logical indicating whether to perform quality/adaptor trimming of reads. See [trimgalore\(\)](#). If TRUE, expects metadata to have a column "fastq_fetched" containing paths to fastq files in `parentDir/study/fetch_output`, saves trimmed files to `parentDir/study/trimgalore_output`, and updates metadata with column "fastq_trimmed". If FALSE, expects and does nothing. Following components are only checked if run is TRUE.
 - keep: Logical indicating whether to keep trimmed fastq files when all processing steps have completed. NULL indicates TRUE.
 - cmd: Name or path of the command-line interface. NULL indicates to use the default in [trimgalore\(\)](#).
 - args: Additional arguments to pass to the command-line interface. NULL indicates to use the default in [trimgalore\(\)](#).
 - pigzCmd: String indicating command for pigz, which converts ".fastq" files to ".fastq.gz" files. NULL indicates to use the default in [trimgalore\(\)](#).

- **fastqc**: Named list with components:
 - **run**: Logical indicating whether to perform QC on reads. See [fastqc\(\)](#). If TRUE and `trimgalore$run` is TRUE, expects metadata to have a column "fastq_trimmed" containing paths to fastq files in `parentDir/study/trimgalore_output`. If TRUE and `trimgalore$run` is FALSE, expects metadata to have a column "fastq_fetched" containing paths to fastq files in `parentDir/study/fetch_output`. If TRUE, saves results to `parentDir/study/fastqc_output`. If FALSE, expects and does nothing. Following components are only checked if `run` is TRUE.
 - **keep**: Logical indicating whether to keep fastqc files when all processing steps have completed. NULL indicates TRUE.
 - **cmd**: Name or path of the command-line interface. NULL indicates to use the default in [fastqc\(\)](#).
 - **args**: Additional arguments to pass to the command-line interface. NULL indicates to use the default in [fastqc\(\)](#).
- **salmon**: Named list with components:
 - **run**: Logical indicating whether to quantify transcript abundances. See [salmon\(\)](#). If TRUE and `trimgalore$run` is TRUE, expects metadata to have a column "fastq_trimmed" containing paths to fastq files in `parentDir/study/trimgalore_output`. If TRUE and `trimgalore$run` is FALSE, expects metadata to have a column "fastq_fetched" containing paths to fastq files in `parentDir/study/fetch_output`. If TRUE, saves results to `parentDir/study/salmon_output` and `parentDir/study/salmon_meta_info.csv`. If FALSE, expects and does nothing. Following components are only checked if `run` is TRUE.
 - **indexDir**: Directory that contains salmon index.
 - **sampleColname**: String indicating column in metadata containing sample ids. NULL indicates "sample_accession", which should work for data from SRA and ENA.
 - **keep**: Logical indicating whether to keep quantification results when all processing steps have completed. NULL indicates TRUE.
 - **cmd**: Name or path of the command-line interface. NULL indicates to use the default in [salmon\(\)](#).
 - **args**: Additional arguments to pass to the command-line interface. NULL indicates to use the default in [salmon\(\)](#).
- **multiqc**: Named list with components:
 - **run**: Logical indicating whether to aggregate results of various processing steps. See [multiqc\(\)](#). If TRUE, saves results to `parentDir/study/multiqc_output`. If FALSE, expects and does nothing. Following components are only checked if `run` is TRUE.
 - **cmd**: Name or path of the command-line interface. NULL indicates to use the default in [multiqc\(\)](#).
 - **args**: Additional arguments to pass to the command-line interface. NULL indicates to use the default in [multiqc\(\)](#).
- **tximport**: Named list with components:

- run: Logical indicating whether to summarize transcript- or gene-level estimates for downstream analysis. See `tximport()`. If TRUE, expects metadata to have a column `sampleColname` of sample ids, and expects a directory `parentDir/study/salmon_output` containing directories of quantification results, and saves results to `parentDir/study/tximport_output.qs`. If FALSE, expects and does nothing. Following components are only checked if run is TRUE.
- tx2gene: Optional named list with components:
 - * `organism`: String indicating organism and thereby ensembl gene dataset. See `getTx2gene()`.
 - * `version`: Optional number indicating ensembl version. NULL indicates the latest version. See `getTx2gene()`.
 - * `filename`: Optional string indicating name of pre-existing text file in `parentDir/params$study` containing mapping between transcripts (first column) and genes (second column), with column names in the first row. If filename is specified, `organism` and `version` must not be specified.
 If not NULL, saves a file `parentDir/study/tx2gene.csv.gz`.
- `countsFromAbundance`: String indicating whether or how to estimate counts using estimated abundances. See `tximport::tximport()`.
- `ignoreTxVersion`: Logical indicating whether to the version suffix on transcript ids. NULL indicates to use TRUE. See `tximport::tximport()`.

`params` can be derived from a yaml file, see `vignette("introduction", package = "seeker")`. The yaml representation of `params` will be saved to `parentDir/params$study/params.yaml`.

<code>parentDir</code>	Directory in which to store the output, which will be a directory named according to <code>params\$study</code> .
<code>dryRun</code>	Logical indicating whether to check the validity of inputs without actually fetching or processing any data.

Value

Path to the output directory `parentDir/params$study`, invisibly.

See Also

`fetchMetadata()`, `fetch()`, `trimgalore()`, `fastqc()`, `salmon()`, `multiqc()`, `tximport()`, `installSysDeps()`, `seekerArray()`

seekerArray

Process microarray data end to end

Description

This function fetches data and metadata from NCBI GEO and ArrayExpress, processes raw Affymetrix data using RMA and custom CDFs from Brainarray, and maps probes to genes.

Usage

```
seekerArray(
  study,
  geneIdType,
  platform = NULL,
  parentDir = ".",
  metadataOnly = FALSE
)
```

Arguments

study	String indicating the study accession and used to name the output directory within parentDir. Must start with "GSE", "E-", or "LOCAL". If starts with "GSE", data are fetched using <code>GEOquery::getGEO()</code> . If starts with "E-", data are fetched using <code>ArrayExpress::getAE()</code> . If starts with "LOCAL", data in the form of <code>cel.gz</code> files must in the directory <code>parentDir/study/raw</code> , and <code>parentDir/study</code> must contain a file "sample_metadata.csv" that has a column <code>sample_id</code> containing the names of the <code>cel.gz</code> files without the file extension.
geneIdType	String indicating whether to map probes to gene IDs from Ensembl ("ensembl") or Entrez ("entrez").
platform	String indicating the GEO-based platform accession for the raw data. See https://www.ncbi.nlm.nih.gov/geo/browse/?view=platforms . Only necessary if study starts with "LOCAL", or starts with "GSE" and the study uses multiple platforms.
parentDir	Directory in which to store the output, which will be a directory named according to study.
metadataOnly	Logical indicating whether to only process the sample metadata, and skip processing the expression data.

Details

The standard output:

- `naive_expression_set.qs`: Initial ExpressionSet generated by `GEOquery::getGEO` or `ArrayExpress::ae2bioc()`. Should generally *not* be used if `sample_metadata.csv` and `gene_expression_matrix.qs` are available.
- `sample_metadata.csv`: Table of sample metadata. Column `sample_id` matches colnames of the gene expression matrix.
- `gene_expression_matrix.qs`: Rows correspond to genes, columns to samples. Expression values are log₂-transformed.
- `custom_cdf_name.txt`: Name of custom CDF package used by `affy::justRMA()` to process and normalize raw Affymetrix data and map probes to genes.
- `feature_metadata.qs`: GPL object, if gene expression matrix was generated from processed data.
- `probe_gene_mapping.csv.gz`: Table of probes and genes, if gene expression matrix was generated from processed data.

- "raw" directory: Contains raw Affymetrix files.
- params.yml: Parameters used to process the dataset.
- session.log: R session information.

The output may include other files from NCBI GEO or ArrayExpress. Files with extension "qs" can be read into R using `qs::qread()`.

Value

Path to the output directory `parentDir/study`, invisibly.

See Also

[seeker\(\)](#)

trimgalore

Run Trim Galore!

Description

This function calls `trim_galore` using `system2()`, and is only designed to handle standard adapter/quality trimming. To run in parallel, register a parallel backend, e.g., using `doParallel::registerDoParallel()`.

Usage

```
trimgalore(
  filepaths,
  outputDir = "trimgalore_output",
  cmd = "trim_galore",
  args = NULL,
  pigzCmd = "pigz"
)
```

Arguments

<code>filepaths</code>	Paths to fastq files. For single-end reads, each element should be a single filepath. For paired-end reads, each element should be two filepaths separated by ";".
<code>outputDir</code>	Directory in which to store output. Will be created if it doesn't exist.
<code>cmd</code>	Name or path of the command-line interface.
<code>args</code>	Additional arguments to pass to the command-line interface. Output files will always be compressed. Arguments <code>"-gzip"</code> , <code>"-cores"</code> , <code>"-j"</code> , and <code>"-basename"</code> are not allowed. Arguments <code>"-o"</code> and <code>"-paired"</code> should not be specified here.
<code>pigzCmd</code>	String for pigz command, which will gzip the output files.

Value

A vector of exit codes, invisibly.

`tximport`*Run tximport on RNA-seq quantifications*

Description

This function uses the [tximport package](#).

Usage

```
tximport(  
  inputDir,  
  tx2gene,  
  samples = NULL,  
  outputDir = "data",  
  type = c("salmon", "kallisto"),  
  countsFromAbundance = "lengthScaledTPM",  
  ignoreTxVersion = TRUE,  
  ...  
)
```

Arguments

<code>inputDir</code>	Directory that contains the quantification directories.
<code>tx2gene</code>	NULL or data.frame of mapping between transcripts and genes, as returned by getTx2gene() , passed to <code>tximport::tximport()</code> .
<code>samples</code>	Names of quantification directories to include. NULL indicates all.
<code>outputDir</code>	Directory in which to save the result, a file named "tximport_output.qs", using qs::qsave() . If NULL, no file is saved.
<code>type</code>	Passed to <code>tximport::tximport()</code> .
<code>countsFromAbundance</code>	Passed to <code>tximport::tximport()</code> .
<code>ignoreTxVersion</code>	Passed to <code>tximport::tximport()</code> .
<code>...</code>	Additional arguments passed to <code>tximport::tximport()</code> .

Value

A list, as returned by `tximport::tximport()`, invisibly.

Index

affy::justRMA(), [15](#)
ArrayExpress::ae2bioc(), [15](#)
ArrayExpress::getAE(), [15](#)

biomaRt::getBM(), [8](#)
biomaRt::useEnsembl(), [8](#)

checkDefaultCommands, [2](#)

doParallel::registerDoParallel(), [3, 4, 10, 16](#)

fastqc, [3](#)
fastqc(), [13, 14](#)
fastqscreen, [4](#)
fastqscreen(), [9](#)
fetch, [4](#)
fetch(), [6, 12, 14](#)
fetchMetadata, [6](#)
fetchMetadata(), [5, 11, 14](#)

GEOquery::getGEO, [15](#)
GEOquery::getGEO(), [15](#)
getPlatforms, [6](#)
getSalmonMetadata, [7](#)
getTx2gene, [7](#)
getTx2gene(), [14, 17](#)

installCustomCdfPackages, [8](#)
installSysDeps, [9](#)
installSysDeps(), [14](#)

multiqc, [10](#)
multiqc(), [13, 14](#)

qs::qread(), [16](#)
qs::qsave(), [17](#)

salmon, [10](#)
salmon(), [9, 13, 14](#)
seeker, [11](#)
seeker(), [9, 16](#)
seekerArray, [14](#)
seekerArray(), [14](#)
system2(), [3, 4, 10, 16](#)

tringalore, [16](#)
tringalore(), [12, 14](#)
tximport, [17](#)
tximport(), [14](#)
tximport::tximport(), [14, 17](#)