Package 'ArrayExpressHTS'

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Title ArrayExpress High Throughput Sequencing Processing Pipeline

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Description RNA-Seq processing pipeline for public ArrayExpress
experiments or local datasets

Depends sampling, Rsamtools (>= 1.19.36), snow

Imports Biobase, BiocGenerics, Biostrings, DESeq, GenomicRanges,
Hmisc, IRanges, R2HTML, RColorBrewer, Rsamtools, ShortRead,
XML, biomaRt, edgeR, grDevices, graphics, methods, rJava,
stats, svMisc, utils, sendmailR, bitops

LinkingTo Rsamtools

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biocViews RNASeq, Sequencing

NeedsCompilation yes

R topics documented:

ArrayExpressHTS   *ExpressionSet for RNA-Seq experiment submitted in ArrayExpress
                                 and ENA*

Description

ArrayExpressHTS runs the RNA-Seq pipeline on a transcription profiling experiment available on
the ArrayExpress database and produces an `ExpressionSet` R object. ArrayExpressHTS requires
an Internet connection.

Usage

```
ArrayExpressHTS( accession,
        options = list (
                stranded          = FALSE,
                insize            = NULL,
                insizedev         = NULL,
                reference         = "genome",
                aligner           = "tophat",
                aligner_options   = NULL,
                count_feature     = "transcript",
                count_options     = "",
                count_method      = "cufflinks",
                filter            = TRUE,
                filtering_options = NULL ),
        usercloud = TRUE,
        rcloudoptions = list (
                nnodes    = "automatic",
                pool      = c("4G", "8G", "16G", "32G", "64G"),
                nretries  = 4 ),
        steplist = c("align", "count", "eset"),
        dir = getwd(),
        refdir = getDefaultReferenceDir(),
        want.reports = TRUE,
        stop.on.warnings = FALSE )
```

Arguments

accession	an ArrayExpress experiment accession identifier, e.g. "E-GEOD-16190"
options	defines pipeline options. See getDefaultProcessingOptions.
usercloud	defines if the R Cloud will be used to parallel experiment computation, if FALSE, experiment data files will be processed sequentially.
rcloudoptions	defines R Cloud options. See getDefaultRCloudOptions.
steplist	defines the steps the pipeline will perform.

dir	folder where experiment data will be stored and processed. Default is current directory.
refdir	the directory where reference data is located.
want.reports	defines if quality reports are produced. Reports usually make computation longer and eat up more memory. For faster computation use FALSE.
stop.on.warnings	
	self explanatory. Warnings are normally producesd when there are inconsistencies, which however would allow the result to be produced.

Value

The output is an object of class [ExpressionSet] containing expression values in assayData (corresponding to the raw sequencing data files), the information contained in the .sdrf file in phenoData, the information in the adf file in featureData and the idf file content in experimentData.

If executed on a local PC, make sure that tools are available to the pipeline. Check [prepareAnnotation] to see what needs to be done to make tools available.

Author(s)

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See Also

[ArrayExpressHTSFastQ], [prepareReference], [prepareAnnotation], [prepareAnnotation] [getDefaultProcessingOption] [getPipelineOptions],

Examples

```
if (isRCloud()) { # disabled on local configs
    # so as not to affect package building process

    # if executed on a local PC, make sure tools
    # are available to the pipeline.
    expfolder = tempdir();

    # run the pipeline
    #
    aehts = ArrayExpressHTS("E-GEOD-16190", dir = expfolder);

    # load the expression set object
    loadednames = load(paste(expfolder,
                        "/E-GEOD-16190/eset_notstd_rpkm.RData", sep=""));
    loadednames;

    get('library')(Biobase);

    # print out the expression values
    #
    head(assayData(eset)$exprs);
```

```
    # print out the experiment meta data
    experimentData(eset);
    pData(eset);
}
```

ArrayExpressHTSFastQ   *ExpressionSet for RNA-Seq raw data files*

Description

ArrayExpressHTSFastQ runs the RNA-Seq pipeline on raw RNA-Seq data files and an .sdrf experiment descriptor and produces an [ExpressionSet](#) R object.

Usage

```
ArrayExpressHTSFastQ( accession,
        organism = c("automatic", "Homo_sapiens", "Mus_musculus" ),
        quality = c("automatic", "FastqQuality", "SFastqQuality"),
        options = list(
                stranded          = FALSE,
                insize            = NULL,
                insizedev         = NULL,
                reference         = "genome",
                aligner           = "tophat",
                aligner_options   = NULL,
                count_feature     = "transcript",
                count_options     = "",
                count_method      = "cufflinks",
                filter            = TRUE,
                filtering_options = NULL),
        usercloud = TRUE,
        rcloudoptions = list(
                nnodes        = "automatic",
                pool          = c("4G", "8G", "16G", "32G", "64G"),
                nretries      = 4),
        steplist = c("align", "count", "eset"),
        dir = getwd(),
        refdir = getDefaultReferenceDir(),
        want.reports = TRUE,
        stop.on.warnings = FALSE )
```

Arguments

accession   name of the folder where experiment data is kept and computaton data is stored.
                 The actual data files and descriptor .sdrf and .idf fles should be stored in the
                 'data' folder, which should be created in this folder.

organism   defines organism filter, "automatic" means all organism found in the descript
                  files will be used. If a specific organism or an array e.g. c("Homo_sapiens",
                  "Mus_musculus") is defined, those organisms will be used to filter out other
                  unwanted organisms and associated data files from the result object. The value
                  canot be "automatic" if no .sdrf is found and no automatic discovery can be
                  performed.

quality   this parameter is used to explicitly select the quality scale used in the fastq data
                  files. By default "automatic" is used. In case quality scale cannot be automati-
                  cally detected, the user will be prompted to increase the detection depth or set the
                  quality scale manually. "FastqQuality" corresponds to Phred+33, "SFastqQual-
                  ity" corresponds to Phred+64.

options   defines pipeline options. See [getDefaultProcessingOptions](#)

usercloud   defines if the R Cloud will be used to parallel experiment computation. If set to
                  FALSE, the experiment files will be processed sequentially.

rcloudoptions   defines R Cloud options. See [getDefaultRCloudOptions](#)

steplist   defines the steps the pipeline will perform

dir   folder where experiment data will be stored and processed. Default is current
                  directory.

refdir   the directory where reference data is located.

want.reports   defines if quality reports are produced. Reports usually make computation longer
                  and eat up more memory. For faster computation use FALSE.

stop.on.warnings
                  self explanatory. Warnings are normally producesd when there are inconsisten-
                  cies, which however would allow the result to be produced.

Value

The output is an object of class [ExpressionSet](#) containing expression values in assayData (corre-
sponding to the raw sequencing data files), the information contained in the .sdrf file in phenoData,
the information in the adf file in featureData and the idf file content in experimentData.

Author(s)

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See Also

[ArrayExpressHTS](#), [prepareReference](#), [prepareAnnotation](#), [prepareAnnotation](#) [getDefaultProcessingOptions](#),
[getPipelineOptions](#),

Examples

```
if (isRCloud()) { # disabled on local configs
    # so as not to affect package building process

    # In ArrayExpressHTS/expdata there is testExperiment, which is
```

```
# a very short version of E-GEOD-16190 experiment, placed there
# for testing reasons.
#
# Experiment in ArrayExpress:
# http://www.ebi.ac.uk/arrayexpress/experiments/E-GEOD-16190
#
# the following piece of code will take ~1.5 hours to compute
# on local PC and ~10 minutes on R-Cloud
#

# if executed on a local PC, make sure tools are available
# to the pipeline.
#

# create a temporary folder where experiment will be copied
# computing experiment in the package folder may cause issues
# with file permissions and therefore failures.
#
#
srcfolder <- system.file("expdata",
                "testExperiment", package="ArrayExpressHTS");

dstfolder <- tempdir();

file.copy(srcfolder, dstfolder, recursive = TRUE);

# run the pipeline
#
# set usercloud = FALSE if executing on local PC,
# therefore parallel computation will be disabled
#
aehts = ArrayExpressHTSFastQ(accession = "testExperiment",
    organism = "Homo_sapiens", dir = dstfolder);

# load the expression set object
loadednames = load(paste(dstfolder,
                    "/testExperiment/eset_notstd_rpkm.RData", sep=""));
loadednames;

get('library')(Biobase);

# print out the expression values
#
head(assayData(eset)$exprs);

# print out the experiment meta data
experimentData(eset);
pData(eset);
}
```

---

isRCloud   *Check the code is running on R-Cloud*

Description

isRCloud returns TRUE/FALSE indicating whether the configuration is an R-Cloud.

Usage

```
isRCloud()
```

Value

If TRUE, the configuration is the R-Cloud.

Author(s)

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See Also

[setPipelineOptions](#), [ArrayExpressHTS](#)

Examples

```
if ( isRCloud() ) {

    # we're on the R-Cloud
    print("R-Cloud configuration");
} else {

    # we're somewhere else
    print("Other configuration");
}
```

---

package-options   *Functions to work with package level options*

Description

Functions to work with ArrayExpressHTS package level options.

Usage

```
getPipelineOptions()
getPipelineOption(name, default = NULL, options = defaultOptions)
setPipelineOptions(...)
```

Arguments

`...`	a list ot options to set.
`name`	name of the option to return. In order to see all options, use [getPipelineOptions](#);
`default`	default values in case the option is not defined
`options`	options environment, by default the ArrayExpresHTS environment;

Details

These are the basic functions to get and set pakcage options and perform necessary reinitialization following the change in required.

getPipelineOptions returns an environment where ArrayExpressHTS options are stored.

setPipelineOptions sets one or a number of ArrayExpressHTS options.

Please note that the following options are used to store locations of external tools used by the package: "ArrayExpressHTS.bowtie", "ArrayExpressHTS.tophat", "ArrayExpressHTS.bwa", "ArrayExpressHTS.cufflinks", "ArrayExpressHTS.mmseq", "ArrayExpressHTS.samtools", etc.

If any of the options needs to be changed, use setPipelineOption(...) to make necessary chnages. Following the change, PATH environment varibale automatically gets updated as necessary.

Please note that the package supports only certain versions of tools. Other versions may not be fully compatible, and if used, this can result in errors on certain steps of the pipeline. The list of supported versions can be obtained using getPieplineOption(...) with the corresponding tool option, e.g. "ArrayExpressHTS.bowtie". Not supported versions can be used at user's own risk.

getPipelineOption returns ArrayExpressHTS option specified by option name. If option is not defined the specified default value will be returned.

Value

The output is the environment where pipeline options are stored.

Author(s)

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See Also

[getDefaultProcessingOptions](#), [ArrayExpressHTS](#), [ArrayExpressHTSFastQ](#)

Examples

```
# get options
pipelineOptions = getPipelineOptions()

# list all names
ls(pipelineOptions)

# set pipeline options
```

```
setPipelineOptions("trace" = "disabled", "memorymonitor" = "disabled");

# get options
getPipelineOption("trace");
getPipelineOption("memorymonitor");
```

---

prepareAnnotation *Prepare annotation data for the RNA-Seq Pipeline*

Description

prepareAnnotation downloads the required annotation file for the selected organism from En-sembl and processes it so that it can be used by the pipeline. prepareAnnotation requires an Internet connection.

Usage

```
prepareAnnotation(organism, version = "current",
    location = getDefaultReferenceDir(), refresh = FALSE, run = TRUE)
```

Arguments

organism	supported organism names can be viewed in the Ensemble database. Check 'ftp://ftp.ensembl.org/pub'.
version	"current" or other appropriate version. Check 'ftp://ftp.ensembl.org/pub'.
location	indicates where the annotation data should be stored.
refresh	if TRUE, existing annotation data will be rebuilt.
run	if FALSE, the commands to obtain and process the annotation will not be executed.

Value

The output is the version of the organism annotation that has been downloaded and processed. The annotation files are kept in the folder defined in location parameter.

Author(s)

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See Also

[ArrayExpressHTS](), [ArrayExpressHTSFastQ](), [prepareReference]()

Examples

```
if (isRCloud()) { # disabled on local configs
    # so as not to affect package building process

    par(ask = FALSE)

    # the following piece of code will take ~1.5 hours to complete
    #

    # if executed on a local PC, make sure tools are available
    # to the pipeline.
    #

    # create directory
    #
    # Please note, tempdir() is used for automamtic test
    # execution. Select directory more appropriate and
    # suitable for keeping reference data.
    #
    referencefolder = paste(tempdir(), "/reference", sep = "")

    dir.create(referencefolder)

    # download and prepare annotation
    prepareAnnotation("Homo_sapiens", "current", location = referencefolder)
    prepareAnnotation("Mus_musculus", "NCBIM37.61", location = referencefolder)

}
```

---

prepareReference   *Prepare reference data for the RNA-Seq Pipeline*

Description

prepareReference downloads reference genome or transcriptome for the selected organism from the Ensembl database and processes it so that it can be used by the pipeline. prepareReference requires an Internet connection.

Usage

```
prepareReference(organism, version = "current",
    type = c("genome", "transcriptome"),
    location = getDefaultReferenceDir(),
    aligner = c("bwa", "bowtie", "tophat"),
    refresh = FALSE, run = TRUE)
```

Arguments

organism	supported organism names can be viewed in the Ensemble database. Check 'ftp://ftp.ensembl.org/pub'.
version	"current" or other appropriate version. Check 'ftp://ftp.ensembl.org/pub'.
type	two values are supported: "genome", "transcriptome"
location	indicates where the reference data should be stored.
aligner	3 types of aligners are supported: "bwa", "bowtie" and "tophat".
refresh	if TRUE, existing reference data will be rebuilt.
run	if FALSE, the downloading and processing commands will not be executed.

Value

The output is the version of the organism reference that has been downloaded and processed. The reference files are kept in the folder defined in location parameter.

Author(s)

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See Also

ArrayExpressHTS, ArrayExpressHTSFastQ, prepareAnnotation

Examples

```
if (isRCloud()) {

    par(ask = FALSE)

    # the following piece of code will take ~3 hours to complete
    #

    # if executed on a local PC, make sure tools are available
    # to the pipeline.
    #

    # create directory
    #
    # Please note, tempdir() is used for automamtic test
    # execution. Select directory more appropriate and
    # suitable for keeping reference data.
    #
    referencefolder = paste(tempdir(), "/reference", sep = "")

    dir.create(referencefolder)

    # download and prepare reference
    prepareReference("Homo_sapiens", version = "GRCh37.61",
```

```
        type = "genome", aligner = "bowtie", location = referencefolder )
    prepareReference("Homo_sapiens", version = "GRCh37.61",
        type = "transcriptome", aligner = "bowtie", location = referencefolder )
    prepareReference("Mus_musculus", version = "current",
        type = "genome", aligner = "bowtie", location = referencefolder )
    prepareReference("Mus_musculus", version = "current",
        type = "transcriptome", aligner = "bowtie", location = referencefolder )
}
```

---

processing-options   *Functions to work with ArrayExpressHTS processing options*

Description

Functions to get default ArrayExpressHTS processing options.

Usage

```
    getDefaultFilteringOptions()
    getDefaultProcessingOptions()
    getDefaultRCloudOptions()
```

Arguments

　　　　　　　　　No arguments.

Value

These functions return default options used for ArrayExpressHTS processing.

Function `getDefaultFilteringOptions` returns a `list` of filtering options. These options control filtering of BAM files after alignemnt.

mismatches	2 by default, number of mismatches
chr_ignore	c("MT") by default, reference names to ignore
minqual	10 by default
minmapq	1 by default
maxN	2 by default
maxpol	0.75 by default, ither a proportion (<0) or an integer
duplicates	"remove" by default, supported value: "keep", "remove"
multihits	"remove" by default, supported value: "keep", "remove"
gapped	"remove" by default, supported value: "keep", "remove"

—

Function `getDefaultProcessingOptions` returns a `list` of options used throught the processing. These options control alignment and counting.

These options include:

stranded	FALSE by default, set to TRUE if a strand specific protocol was used
insize	NULL by default, the insert size, an integer, which will be automatically determined if set to NULL
insizedev	NULL by default, insert size deviation, an integer, which will be automatically determined if set to NULL
reference	"genome" by default, controlls whether alignment is performed using "genome" or "transcriptome" reference.
aligner	"tophat" by default, supported values are: "tophat", "bowtie", "bwa" or "custom"
aligner_options	
	NULL by default, string of options to be passed to the aligner according to manual.
count_feature	"transcript" by default, count over "gene" or "transcript"
count_method	"cufflinks" by default, supported: "cufflinks", "mmseq" or "count"
count_options	options for count software
standardise	FALSE by default, supported: TRUE, FALSE
normalisation	"rpkm" by default, supported: "none" or "rpkm"
filter	"TRUE" by defult, supported: "TRUE", "FALSE"
filtering_options	
	these are the default filtering options, see above.

—

Function getDefaultRCloudOptions returns a set of R Cloud options related to R Cloud resources and execution flow.

These include:

nnodes	"automatic" by default, supported: "automatic" or a numeric value, e.g. 10
pool	"32G" by default, supported: "4G", "8G", "16G", "32G", "64G"
nretries	4 by default, a numberic value.

Author(s)

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See Also

getPipelineOptions, ArrayExpressHTS, ArrayExpressHTSFastQ

Examples

```
# get filtering options
getDefaultFilteringOptions()

# get alignment and counting options
getDefaultProcessingOptions()
```

```
# get R Cloud options
getDefaultRCloudOptions()
```

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