Package 'RNASeqR'

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Type Package

Title RNASeqR: an R package for automated two-group RNA-Seq analysis
workflow

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GeneExpression, GeneSetEnrichment, Alignment, QualityControl,
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ExperimentalDesign, GO, KEGG, Visualization, Normalization,
Pathways, Clustering, ImmunoOncology

Description This R package is designed for case-control RNA-Seq analysis (two-group).
There are six steps: ``RNASeqRParam S4 Object Creation'', ``Environment Setup'', ``Quality Assessment'',
``Reads Alignment & Quantification'', ``Gene-level Differential Analyses'' and ``Functional Analyses''.
Each step corresponds to a function in this package. After running functions in order,
a basic RNASeq analysis would be done easily.

License Artistic-2.0

Encoding UTF-8

RoxygenNote 6.1.1

Depends R(>= 3.5.0), ggplot2, pathview, edgeR, methods

Imports Rsamtools, tools, reticulate, ballgown, gridExtra, rafalib,
FactoMineR, factoextra, corrplot, PerformanceAnalytics,
reshape2, DESeq2, systemPipeR, systemPipeRdata,
clusterProfiler, org.Hs.eg.db, org.Sc.sgd.db, stringr,
pheatmap, grDevices, graphics, stats, utils, DOSE, Biostrings,
parallel

Suggests knitr, rmarkdown, png, grid, RNASeqRData

VignetteBuilder knitr

SystemRequirements RNASeqR only support Linux and macOS. Window is not supported. Python2 is highly recommended. If your machine is Python3, make sure '2to3' command is available.

BugReports <https://github.com/HowardChao/RNASeqR/issues>

URL <https://github.com/HowardChao/RNASeqR>

NeedsCompilation no

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

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R **topics documented:**

`All_Steps_Interface`   *RNASeqEnvironmentSet*

Description

Set up the environment for the following RNA-Seq workflow in R shell
This function do 4 things :

1. Create file directories.

2. Install necessary tools.

3. Export 'RNASeq_bin/' to the R environment.

4. Check command of tools.

First it will create 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/', 'Rscript_out/' directories.
Afterwards, 'Hisat2', 'Stringtie', 'Gffcompare' will be installed under 'RNASeq_bin/Download/'
and be unpacked under 'RNASeq_bin/Unpacked/'.
'RNASeq_bin/' will be added to the R environment and validity of tools will be checked.
Any ERROR occurs will be reported and the program will be terminated.
If you want to set up the environment for the following RNA-Seq workflow in background, please
see RNASeqEnvironmentSet_CMD() function.

Usage

```
All_Steps_Interface(RNASeqRParam, which.trigger = "OUTSIDE",
  INSIDE.path.prefix = NA, RNASeqQualityAssessment.RUN = TRUE,
  RNASeqReadProcess.RUN = TRUE, RNASeqDifferentialAnalysis.RUN = TRUE,
  RNASeqGoKegg.RUN = TRUE, OrgDb.species, go.level = 3, input.TYPE.ID,
  KEGG.organism, check.s4.print = TRUE)
```

Arguments

RNASeqRParam   S4 object instance of experiment-related parameters

which.trigger   Default value is `OUTSIDE`. User should not change this value.

INSIDE.path.prefix
                Default value is `NA`. User should not change this value.

RNASeqQualityAssessment.RUN
                Default value is `TRUE`. Set `FALSE` to skip "Quality Assessment" step.

RNASeqReadProcess.RUN
                Default value is `TRUE`. Set `FALSE` to skip "RNASeq Read Process" step.

RNASeqDifferentialAnalysis.RUN

> Default value is `TRUE`. Set `FALSE` to skip "RNASeq Differential Analysis" step.

RNASeqGoKegg.RUN

> Default value is `TRUE`. Set `FALSE` to skip "RNASeq Go & Kegg" step.

OrgDb.species   the genome wide annotation packages of species on Bioconductor. Currently, there are 19 supported genome wide annotation packages of species.

go.level   the depth of acyclic graph in GO analysis

input.TYPE.ID   The gene name type in OrgDb.species annotation packahge.

KEGG.organism   the species that are supported for KEGG analysis. Currently, there are more than 5000 supported species genome. Check the valid species terms on https://www.genome.jp/kegg/catalog/or

check.s4.print Default `TRUE`. If `TRUE`, the result of checking `RNASeqRParam` will be reported in 'Rscript_out/Environment_Set.Rout'. If `FALSE`, the result of checking `RNASeqRParam` will not be in 'Rscript_out/Environment_Set.Rout'.

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqEnvironmentSet(RNASeqRParam = yeast)
## End(Not run)
```

All_Steps_Interface_CMD

All_Steps_Interface_CMD

Description

A functios to run all the steps with in one function. This function execute in the background:

1. Create file directories.

2. Install necessary tools.

3. Export 'RNASeq_bin/' to the R environment.

4. Check command of tools.

First it will create 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/', 'Rscript_out/' directories.

Afterwards, 'Hisat2', 'Stringtie', 'Gffcompare' will be installed under 'RNASeq_bin/Download/' and be unpacked under 'RNASeq_bin/Unpacked/'.

'RNASeq_bin/' will be added to the R environment and validity of tools will be checked.

Any ERROR occurs will be reported and the program will be terminated.

If you want to set up the environment for the following RNA-Seq workflow in R shell, please see RNASeqEnvironmentSet() function.

Usage

```
All_Steps_Interface_CMD(RNASeqRParam, RNASeqQualityAssessment.RUN = TRUE,
  RNASeqReadProcess.RUN = TRUE, RNASeqDifferentialAnalysis.RUN = TRUE,
  RNASeqGoKegg.RUN = TRUE, OrgDb.species, go.level = 3, input.TYPE.ID,
  KEGG.organism, run = TRUE, check.s4.print = TRUE)
```

Arguments

RNASeqRParam   S4 object instance of experiment-related parameters

RNASeqQualityAssessment.RUN
                   Default value is TRUE. Set FALSE to skip "Quality Assessment" step.

RNASeqReadProcess.RUN
                   Default value is TRUE. Set FALSE to skip "RNASeq Read Process" step.

RNASeqDifferentialAnalysis.RUN
                   Default value is TRUE. Set FALSE to skip "RNASeq Differential Analysis" step.

RNASeqGoKegg.RUN
                   Default value is TRUE. Set FALSE to skip "RNASeq Go & Kegg" step.

OrgDb.species   the genome wide annotation packages of species on Bioconductor. Currently, there are 19 supported genome wide annotation packages of species.

go.level   the depth of acyclic graph in GO analysis

input.TYPE.ID   The gene name type in OrgDb.species annotation packahge.

KEGG.organism   the species that are supported for KEGG analysis. Currently, there are more than 5000 supported species genome. Check the valid species terms on https://www.genome.jp/kegg/catalog/or

run   Default value is TRUE. If TRUE, 'Rscript/Environment_Set.R' will be created and executed. The output log will be stored in 'Rscript_out/Environment_Set.Rout'. If False, 'Rscript/Environment_Set.R' will be created without executed.

check.s4.print   Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in 'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking RNASeqRParam will not be in 'Rscript_out/Environment_Set.Rout'.

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqEnvironmentSet_CMD(yeast)
## End(Not run)
```

CheckToolAll   *CheckToolAll*

Description

Check whether 'Hisat2', 'Stringtie' and 'Gffcompare' are installed on the workstation

Usage

```
CheckToolAll(path.prefix, print = TRUE)
```

Arguments

path.prefix	path prefix of 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/' and 'Rscript_out/' directories.
print	If TRUE, detailed information will be printed. If FALSE, detailed information will not be printed.

Value

None

Examples

```
data(yeast)
## Not run:
CheckToolAll(yeast@path.prefix,
             print=TRUE)
## End(Not run)
```

RNASeqDifferentialAnalysis

RNASeqDifferentialAnalysis

Description

This function will run differential analysis on ballgown, DESeq2 and edgeR in background.
This function do following things :

1. ballgown analysis
   Raw reads are normalized into FPKM values
   The main statistic test in ballgown is paramatic F-test comparing nested linear models

2. DESeq2 analysis
   Median of rations normalization(MRN) is used in DESeq2 for raw reads count normalization.
   Sequencing depth and RNA composition is taken into consideration is this normalization
   method.
   The main statistic test in DESeq2 is negative binomial distribution.

3. edgeR analysis
   Raw reads are normalized by TMM and library size. (run `calcNormFactors()` to get a DGE-
   List, and then run `cpm()` on that DGEList)
   The main statistic test in edgeR is trimmed mean of M-values(TMM).

If you want to run differential analysis on ballgown, DESeq2, edgeR for the following RNA-Seq
workflow in background, please see `RNASeqDifferentialAnalysis()` function.

Usage

```
RNASeqDifferentialAnalysis(RNASeqRParam, which.trigger = "OUTSIDE",
  INSIDE.path.prefix = NA, Pre_DE.visualization = TRUE,
  Post_DE.visualization = TRUE, ballgown.run = TRUE,
  ballgown.pval = 0.05, ballgown.log2FC = 1, DESeq2.run = TRUE,
  DESeq2.pval = 0.1, DESeq2.log2FC = 1, edgeR.run = TRUE,
  edgeR.pval = 0.05, edgeR.log2FC = 1, check.s4.print = TRUE)
```

Arguments

`RNASeqRParam`	S4 object instance of experiment-related parameters
`which.trigger`	Default value is `OUTSIDE`. User should not change this value.
`INSIDE.path.prefix`	
	Default value is `NA`. User should not change this value.
`Pre_DE.visualization`	
	Default `TRUE`. Whether to visualize pre-DE analysis results.

Post_DE.visualization

        Default TRUE. Whether to visualize post-DE analysis results.

ballgown.run   Default TRUE. Logical value whether to run ballgown differential analysis.

ballgown.pval   Default 0.05. Set the threshold of ballgown p-value to filter out differential expressed gene.

ballgown.log2FC

        Default 1. Set the threshold of ballgown log2 fold change to filter out differential expressed gene.

DESeq2.run   Default TRUE. Logical value whether to run DESeq2 differential analysis.

DESeq2.pval   Default 0.05. Set the threshold of DESeq2 p-value to filter out differential expressed gene.

DESeq2.log2FC   Default 1. Set the threshold of DESeq2 log2 fold change to filter out differential expressed gene.

edgeR.run   Default TRUE. Logical value whether to run edgeR differential analysis.

edgeR.pval   Default 0.05. Set the threshold of edgeR p-value to filter out differential expressed gene.

edgeR.log2FC   Default 1. Set the threshold of edgeR log2 fold change to filter out differential expressed gene.

check.s4.print Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in 'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking RNASeqRParam will not be in 'Rscript_out/Environment_Set.Rout'.

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqDifferentialAnalysis(RNASeqRParam = yeast)
## End(Not run)
```

RNASeqDifferentialAnalysis_CMD

RNASeqDifferentialAnalysis_CMD

Description

This function will run differential analysis on ballgown, DESeq2 and edgeR in R shell.
This function do following things :

1. ballgown analysis
   Raw reads are normalized into FPKM values
   The main statistic test in ballgown is paramatic F-test comparing nested linear models

2. DESeq2 analysis
   Median of rations normalization(MRN) is used in DESeq2 for raw reads count normalization.
   Sequencing depth and RNA composition is taken into consideration is this normalization
   method.
   The main statistic test in DESeq2 is negative binomial distribution.

3. edgeR analysis
   Raw reads are normalized by TMM and library size. (run `calcNormFactors()` to get a DGE-
   List, and then run `cpm()` on that DGEList)
   The main statistic test in edgeR is trimmed mean of M-values(TMM).

If you want to run differential analysis on ballgown, DESeq2, edgeR for the following RNA-Seq
workflow in R shell, please see `RNASeqDifferentialAnalysis()` function.

Usage

```
RNASeqDifferentialAnalysis_CMD(RNASeqRParam, which.trigger = "OUTSIDE",
  INSIDE.path.prefix = NA, Pre_DE.visualization = TRUE,
  Post_DE.visualization = TRUE, ballgown.run = TRUE,
  ballgown.pval = 0.05, ballgown.log2FC = 1, DESeq2.run = TRUE,
  DESeq2.pval = 0.1, DESeq2.log2FC = 1, edgeR.run = TRUE,
  edgeR.pval = 0.05, edgeR.log2FC = 1, run = TRUE,
  check.s4.print = TRUE)
```

Arguments

RNASeqRParam	S4 object instance of experiment-related parameters
which.trigger	Default value is `OUTSIDE`. User should not change this value.
INSIDE.path.prefix	
	Default value is `NA`. User should not change this value.
Pre_DE.visualization	
	Default `TRUE`. Whether to visualize pre-DE analysis results.
Post_DE.visualization	
	Default `TRUE`. Whether to visualize post-DE analysis results.
ballgown.run	Default `TRUE`. Logical value whether to run ballgown differential analysis.
ballgown.pval	Default `0.05`. Set the threshold of ballgown p-value to filter out differential expressed gene.

ballgown.log2FC
> Default 1. Set the threshold of ballgown log2 fold change to filter out differential expressed gene.

DESeq2.run Default TRUE. Logical value whether to run DESeq2 differential analysis.

DESeq2.pval Default `0.05`. Set the threshold of DESeq2 p-value to filter out differential expressed gene.

DESeq2.log2FC Default 1. Set the threshold of DESeq2 log2 fold change to filter out differential expressed gene.

edgeR.run Default TRUE. Logical value whether to run edgeR differential analysis.

edgeR.pval Default `0.05`. Set the threshold of edgeR p-value to filter out differential expressed gene.

edgeR.log2FC Default 1. Set the threshold of edgeR log2 fold change to filter out differential expressed gene.

run Default value is TRUE. If TRUE, 'Rscript/Environment_Set.R' will be created and executed. The output log will be stored in 'Rscript_out/Environment_Set.Rout'. If `False`, 'Rscript/Environment_Set.R' will be created without executed.

check.s4.print Default TRUE. If TRUE, the result of checking `RNASeqRParam` will be reported in 'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking `RNASeqRParam` will not be in 'Rscript_out/Environment_Set.Rout'.

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqDifferentialAnalysis_CMD(RNASeqRParam = yeast)
## End(Not run)
```

RNASeqEnvironmentSet *RNASeqEnvironmentSet*

Description

Set up the environment for the following RNA-Seq workflow in R shell
This function do 4 things :

1. Create file directories.

2. Install necessary tools.

3. Export 'RNASeq_bin/' to the R environment.

4. Check command of tools.

First it will create 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/', 'Rscript_out/' directories.
Afterwards, 'Hisat2', 'Stringtie', 'Gffcompare' will be installed under 'RNASeq_bin/Download/' and be unpacked under 'RNASeq_bin/Unpacked/'.
'RNASeq_bin/' will be added to the R environment and validity of tools will be checked.
Any ERROR occurs will be reported and the program will be terminated.
If you want to set up the environment for the following RNA-Seq workflow in background, please see RNASeqEnvironmentSet_CMD() function.

Usage

```
RNASeqEnvironmentSet(RNASeqRParam, which.trigger = "OUTSIDE",
  INSIDE.path.prefix = NA, install.hisat2 = TRUE,
  install.STAR = TRUE, install.stringtie = TRUE,
  install.gffcompare = TRUE, check.s4.print = TRUE)
```

Arguments

RNASeqRParam   S4 object instance of experiment-related parameters

which.trigger   Default value is OUTSIDE. User should not change this value.

INSIDE.path.prefix
                    Default value is NA. User should not change this value.

install.hisat2   Whether to install 'HISAT2' in this function step. Default value isTRUE. Set
                    FALSE to skip 'HISAT2' installation.

install.STAR   Whether to install 'STAR' in this function step. Default value isTRUE. Set FALSE
                    to skip 'STAR' installation.

install.stringtie
                    Whether to install 'StringTie' in this function step. Default value is TRUE.
                    SetFALSE to skip 'StringTie' installation.

install.gffcompare
                    Whether to install 'Gffcompare' in this function step. Default value is TRUE.
                    SetFALSE to skip 'Gffcompare' installation.

check.s4.print   Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in
                    'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking RNASeqRParam
                    will not be in 'Rscript_out/Environment_Set.Rout'.

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqEnvironmentSet(RNASeqRParam = yeast)
## End(Not run)
```

RNASeqEnvironmentSet_CMD

RNASeqEnvironmentSet_CMD

Description

Set up the environment for the following RNA-Seq workflow in background.
This function do 4 things :

1. Create file directories.

2. Install necessary tools.

3. Export 'RNASeq_bin/' to the R environment.

4. Check command of tools.

First it will create 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/', 'Rscript_out/' directories.
Afterwards, 'Hisat2', 'Stringtie', 'Gffcompare' will be installed under 'RNASeq_bin/Download/' and be unpacked under 'RNASeq_bin/Unpacked/'.
'RNASeq_bin/' will be added to the R environment and validity of tools will be checked.
Any ERROR occurs will be reported and the program will be terminated.
If you want to set up the environment for the following RNA-Seq workflow in R shell, please see RNASeqEnvironmentSet() function.

Usage

```
RNASeqEnvironmentSet_CMD(RNASeqRParam, install.hisat2 = TRUE,
    install.STAR = TRUE, install.stringtie = TRUE,
    install.gffcompare = TRUE, run = TRUE, check.s4.print = TRUE)
```

Arguments

RNASeqRParam	S4 object instance of experiment-related parameters
install.hisat2	Whether to install 'HISAT2' in this function step. Default value isTRUE. Set FALSE to skip 'HISAT2' installation.
install.STAR	Whether to install 'STAR' in this function step. Default value isTRUE. Set FALSE to skip 'STAR' installation.
install.stringtie	
	Whether to install 'StringTie' in this function step. Default value is TRUE. SetFALSE to skip 'StringTie' installation.
install.gffcompare	
	Whether to install 'Gffcompare' in this function step. Default value is TRUE. SetFALSE to skip 'Gffcompare' installation.
run	Default value is TRUE. If TRUE, 'Rscript/Environment_Set.R' will be created and executed. The output log will be stored in 'Rscript_out/Environment_Set.Rout'. If False, 'Rscript/Environment_Set.R' will be created without executed.
check.s4.print	Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in 'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking RNASeqRParam will not be in 'Rscript_out/Environment_Set.Rout'.

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqEnvironmentSet_CMD(yeast)
## End(Not run)
```

RNASeqGoKegg *RNASeqGoKegg*

Description

Run Gene Ontology(GO) and Kyoto Encyclopedia of Genes and Genomes(KEGG) analysis in R shell.
This function do Gene Ontology(GO) and Kyoto Encyclopedia of Genes and Genomes(KEGG) analysis :

1. Gene Ontology(GO) :

    (a) Do GO function classification analysis.

    (b) Do GO function enrichment analysis.

    (c) Visualization : bar plot, dot plot etc.

2. Kyoto Encyclopedia of Genes and Genomes(KEGG) :

    (a) Do KEGG pathway enrichment analysis

    (b) Pathway visulization with `pathview` package. KEGG webpage pathway url will also be created

If you want to do GO functional analysis and KEGG pathway analysis for the following RNA-Seq workflow in background, please see RNASeqGoKegg_CMD() function.

Usage

```
RNASeqGoKegg(RNASeqRParam, which.trigger = "OUTSIDE",
  INSIDE.path.prefix = NA, OrgDb.species, go.level = 3, input.TYPE.ID,
  KEGG.organism, check.s4.print = TRUE)
```

Arguments

RNASeqRParam	S4 object instance of experiment-related parameters
which.trigger	Default value is OUTSIDE. User should not change this value.
INSIDE.path.prefix	
	Default value is NA. User should not change this value.
OrgDb.species	the genome wide annotation packages of species on Bioconductor. Currently, there are 19 supported genome wide annotation packages of species.
go.level	the depth of acyclic graph in GO analysis
input.TYPE.ID	The gene name type in OrgDb.species annotation packahge.
KEGG.organism	the species that are supported for KEGG analysis. Currently, there are more than 5000 supported species genome. Check the valid species terms on https://www.genome.jp/kegg/catalog/or
check.s4.print	Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in 'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking RNASeqRParam will not be in 'Rscript_out/Environment_Set.Rout'

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqGoKegg(RNASeqRParam  = yeast,
             OrgDb.species = "org.Sc.sgd.db",
             go.level = 3,
             input.TYPE.ID = "GENENAME",
             KEGG.organism = "sce")
## End(Not run)
```

RNASeqGoKegg_CMD   *RNASeqGoKegg_CMD*

Description

Run Gene Ontology(GO) and Kyoto Encyclopedia of Genes and Genomes(KEGG) analysis in background.
This function do Gene Ontology(GO) and Kyoto Encyclopedia of Genes and Genomes(KEGG) analysis :

1. Gene Ontology(GO) :

   (a) Do GO function classification analysis.

   (b) Do GO function enrichment analysis.

   (c) Visualization : bar plot, dot plot etc.

2. Kyoto Encyclopedia of Genes and Genomes(KEGG) :

   (a) Do KEGG pathway enrichment analysis

   (b) Pathway visulization with pathview package. KEGG webpage pathway url will also be created

If you want to do GO functional analysis and KEGG pathway analysis for the following RNA-Seq workflow in R shell, please see RNASeqGoKegg() function.

Usage

```
RNASeqGoKegg_CMD(RNASeqRParam, OrgDb.species, go.level = 3,
  input.TYPE.ID, KEGG.organism, run = TRUE, check.s4.print = TRUE)
```

Arguments

`RNASeqRParam`	S4 object instance of experiment-related parameters
`OrgDb.species`	the genome wide annotation packages of species on Bioconductor. Currently, there are 19 supported genome wide annotation packages of species.
`go.level`	the depth of acyclic graph in GO analysis
`input.TYPE.ID`	The gene name type in OrgDb.species annotation packahge.
`KEGG.organism`	the species that are supported for KEGG analysis. Currently, there are more than 5000 supported species genome. Check the valid species terms on https://www.genome.jp/kegg/catalog/or
`run`	Default value is TRUE. If TRUE, 'Rscript/Environment_Set.R' will be created and executed. The output log will be stored in 'Rscript_out/Environment_Set.Rout'. If `False`, 'Rscript/Environment_Set.R' will be created without executed.
`check.s4.print`	Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in 'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking `RNASeqRParam` will not be in 'Rscript_out/Environment_Set.Rout'

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqGoKegg_CMD(RNASeqRParam  = yeast,
                 OrgDb.species = "org.Sc.sgd.db",
                 go.level = 3,
                 input.TYPE.ID = "GENENAME",
                 KEGG.organism = "sce")

## End(Not run)
```

RNASeqQualityAssessment

RNASeqQualityAssessment

Description

Assess the quality of '.fastq.gz' files for RNA-Seq workflow in R shell. This step is optional in the whole RNA-Seq workflow.

This function reports the quality assessment result in packages systemPipeR For systemPipeR, 'RNASeq_results/QA_results/Rqc/systemPipeR/fastqReport.pdf' will be created.

If you want to assess the quality of '.fastq.gz' files for the following RNA-Seq workflow in background, please see RNASeqQualityAssessment_CMD() function.

Usage

```
RNASeqQualityAssessment(RNASeqRParam, which.trigger = "OUTSIDE",
    INSIDE.path.prefix = NA, check.s4.print = TRUE)
```

Arguments

RNASeqRParam   S4 object instance of experiment-related parameters

which.trigger   Default value is OUTSIDE. User should not change this value.

INSIDE.path.prefix
                    Default value is NA. User should not change this value.

check.s4.print   Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in
                    'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking RNASeqRParam
                    will not be in 'Rscript_out/Environment_Set.Rout'

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqQualityAssessment(RNASeqRParam = yeast)
## End(Not run)
```

RNASeqQualityAssessment_CMD

RNASeqQualityAssessment_CMD

Description

Assess the quality of '.fastq.gz' files for RNA-Seq workflow in background. This step is optional
in the whole RNA-Seq workflow.

This function reports the quality assessment result in packages systemPipeR For systemPipeR,
'RNASeq_results/QA_results/Rqc/systemPipeR/fastqReport.pdf' will be created.

If you want to assess the quality of '.fastq.gz' files for the following RNA-Seq workflow in R shell,
please see RNASeqQualityAssessment() function.

Usage

```
RNASeqQualityAssessment_CMD(RNASeqRParam, run = TRUE,
    check.s4.print = TRUE)
```

Arguments

`RNASeqRParam`	S4 object instance of experiment-related parameters
`run`	Default value is TRUE. If TRUE, 'Rscript/Environment_Set.R' will be created and executed. The output log will be stored in 'Rscript_out/Environment_Set.Rout'. If `False`, 'Rscript/Environment_Set.R' will be created without executed.
`check.s4.print`	Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in 'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking `RNASeqRParam` will not be in 'Rscript_out/Environment_Set.Rout'

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqQualityAssessment_CMD(RNASeqRParam = yeast)
## End(Not run)
```

RNASeqR	*RNASeqR-package*

Description

RNASeqR-package

RNASeqReadProcess	*RNASeqReadProcess*

Description

Process raw reads for RNA-Seq workflow in R shell
This function do 5 things :

1. 'Hisat2' : aligns raw reads to reference genome. If `indices.optional` in `RNASeqRParam` is FALSE, Hisat2 indices will be created.

2. 'Rsamtools': converts '.sam' files to '.bam' files.

3. 'Stringtie': assembles alignments into transcript.

4. 'Gffcompare': examines how transcripts compare with the reference annotation.

5. 'Stringtie': creates input files for ballgown, edgeR and DESeq2.

6. raw reads count: create raw reads count for DESeq2 and edgeR

Before running this function, RNASeqEnvironmentSet_CMD() or RNASeqEnvironmentSet() must be executed successfully. If you want to process raw reads for the following RNA-Seq workflow in background, please see RNASeqReadProcess_CMD() function.

Usage

```
RNASeqReadProcess(RNASeqRParam, which.trigger = "OUTSIDE",
  INSIDE.path.prefix = NA, SAMtools.or.Rsamtools = "Rsamtools",
  Hisat2.Index.run = TRUE, Hisat2.Index.num.parallel.threads = "1",
  Hisat2.Index.large.index = FALSE,
  Hisat2.Index.local.ftab.chars = "6",
  Hisat2.Index.local.off.rate = "3", Hisat2.Index.ftab.chars = "10",
  Hisat2.Index.off.rate = "4", Hisat2.Alignment.run = TRUE,
  Hisat2.Alignment.num.parallel.threads = "1",
  Hisat2.Alignment.skip = "0", Hisat2.Alignment.trim5 = "0",
  Hisat2.Alignment.trim3 = "0",
  Hisat2.Alignment.n.ceil.1.function.type = "L",
  Hisat2.Alignment.n.ceil.2.constant.term = "0",
  Hisat2.Alignment.n.ceil.3.coefficient = "0.15",
  Hisat2.Alignment.mp.MX = "6", Hisat2.Alignment.mp.MN = "2",
  Hisat2.Alignment.sp.MX = "2", Hisat2.Alignment.sp.MN = "1",
  Hisat2.Alignment.np = "1", Hisat2.Alignment.rdg.1 = "5",
  Hisat2.Alignment.rdg.2 = "3", Hisat2.Alignment.rfg.1 = "5",
  Hisat2.Alignment.rfg.2 = "3",
  Hisat2.Alignment.score.min.1.function.type = "L",
  Hisat2.Alignment.score.min.2.constant.term = "0",
  Hisat2.Alignment.score.min.3.coefficient = "-0.2",
  Hisat2.Alignment.pen.cansplice = "0",
  Hisat2.Alignment.penc.noncansplice = "12",
  Hisat2.Alignment.pen.canintronlen.1.function.type = "G",
  Hisat2.Alignment.pen.canintronlen.2.constant.term = "-8",
  Hisat2.Alignment.pen.canintronlen.3.coefficient = "1",
  Hisat2.Alignment.pen.noncanintronlen.1.function.type = "G",
  Hisat2.Alignment.pen.noncanintronlen.2.constant.term = "-8",
  Hisat2.Alignment.pen.noncanintronlen.3.coefficient = "1",
  Hisat2.Alignment.min.intronlen = "20",
  Hisat2.Alignment.max.intronlen = "500000",
  Hisat2.Alignment.rna.strandness = "None", Hisat2.Alignment.k = "5",
  Hisat2.Alignment.max.seeds = "5", Hisat2.Alignment.secondary = FALSE,
```

```
Hisat2.Alignment.minins = "0", Hisat2.Alignment.maxins = "500",
Hisat2.Alignment.seed = "0", STAR.Index.num.parallel.threads = "1",
STAR.Index.sjdbOverhang.Read.length = "100",
STAR.Index.genomeSAindexNbases = "14",
STAR.Index.genomeChrBinNbits = "18",
STAR.Index.genomeSAsparseD = "1", STAR.Alignment.run = FALSE,
STAR.Alignment.num.parallel.threads = "1",
STAR.Alignment.genomeLoad = "NoSharedMemory",
STAR.Alignment.readMapNumber = "-1",
STAR.Alignment.clip3pNbases = "0", STAR.Alignment.clip5pNbases = "0",
STAR.Alignment.clip3pAdapterSeq = "-",
STAR.Alignment.clip3pAdapterMMp = "0.1",
STAR.Alignment.clip3pAfterAdapterNbases = "0",
STAR.Alignment.limitGenomeGenerateRAM = "31000000000",
STAR.Alignment.limitIObufferSize = "150000000",
STAR.Alignment.limitOutSAMoneReadBytes = "100000",
STAR.Alignment.limitOutSJoneRead = "1000",
STAR.Alignment.limitOutSJcollapsed = "1000000",
STAR.Alignment.limitBAMsortRAM = "0",
STAR.Alignment.outReadsUnmapped = "None",
STAR.Alignment.outQSconversionAdd = "0",
STAR.Alignment.outSAMprimaryFlag = "OneBestScore",
STAR.Alignment.outSAMmapqUnique = "255",
STAR.Alignment.scoreGap = "0", STAR.Alignment.scoreGapNoncan = "-8",
STAR.Alignment.scoreGapGCAG = "-4",
STAR.Alignment.scoreGapATAC = "-8",
STAR.Alignment.scoreGenomicLengthLog2scale = "-0.25",
STAR.Alignment.scoreDelOpen = "-2",
STAR.Alignment.scoreDelBase = "-2",
STAR.Alignment.scoreInsOpen = "-2",
STAR.Alignment.scoreInsBase = "-2",
STAR.Alignment.scoreStitchSJshift = "1",
STAR.Alignment.seedSearchStartLmax = "50",
STAR.Alignment.seedSearchStartLmaxOverLread = "1.0",
STAR.Alignment.seedSearchLmax = "0",
STAR.Alignment.seedMultimapNmax = "10000",
STAR.Alignment.seedPerReadNmax = "1000",
STAR.Alignment.seedPerWindowNmax = "50",
STAR.Alignment.seedNoneLociPerWindow = "10",
STAR.Alignment.alignIntronMin = "21",
STAR.Alignment.alignIntronMax = "0",
STAR.Alignment.alignMatesGapMax = "0",
STAR.Alignment.alignSJoverhangMin = "5",
STAR.Alignment.alignSJDBoverhangMin = "3",
STAR.Alignment.alignSplicedMateMapLmin = "0",
STAR.Alignment.alignSplicedMateMapLminOverLmate = "0.66",
STAR.Alignment.alignWindowsPerReadNmax = "10000",
STAR.Alignment.alignTranscriptsPerWindowNmax = "100",
```

```
        STAR.Alignment.alignTranscriptsPerReadNmax = "10000",
        STAR.Alignment.alignEndsType = "Local",
        STAR.Alignment.winAnchorMultimapNmax = "50",
        STAR.Alignment.winBinNbits = "16",
        STAR.Alignment.winAnchorDistNbins = "9",
        STAR.Alignment.winFlankNbins = "4", Rsamtools.Bam.run = TRUE,
        Samtools.Bam.num.parallel.threads = "1", Rsamtools.nCores = "1",
        StringTie.Assemble.run = TRUE,
        Stringtie.Assembly.num.parallel.threads = "1",
        Stringtie.Assembly.f = "0.1", Stringtie.Assembly.m = "200",
        Stringtie.Assembly.c = "2.5", Stringtie.Assembly.g = "50",
        Stringtie.Assembly.M = "0.95", StringTie.Merge.Trans.run = TRUE,
        Stringtie.Merge.num.parallel.threads = "1",
        Gffcompare.Ref.Sample.run = TRUE, StringTie.Ballgown.run = TRUE,
        Stringtie.2.Ballgown.num.parallel.threads = "1",
        PreDECountTable.run = TRUE, check.s4.print = TRUE)
```

Arguments

RNASeqRParam   S4 object instance of experiment-related parameters

which.trigger   Default value is OUTSIDE. User should not change this value.

INSIDE.path.prefix
                  Default value is NA. User should not change this value.

SAMtools.or.Rsamtools
                  Default value is Rsamtools. User can set to SAMtools to use command-line-
                  based 'samtools' instead.

Hisat2.Index.run
                  Whether to run 'HISAT2 index' step in this function step. Default value is TRUE.
                  Set FALSE to skip 'HISAT2 index' step.

Hisat2.Index.num.parallel.threads
                  Specify the number of processing threads (CPUs) to use for Hisat2 index step.
                  The default is "1"

Hisat2.Index.large.index
                  Hisat2 index terminal '–large-index' option. Default value is FALSE

Hisat2.Index.local.ftab.chars
                  Hisat2 index terminal '-t/–ftabchars' option. Default value is "6"

Hisat2.Index.local.off.rate
                  Hisat2 index terminal '–localoffrate' option. Default value is "3"

Hisat2.Index.ftab.chars
                  Hisat2 index terminal '–localftabchars' option. Default value is "10"

Hisat2.Index.off.rate
                  Hisat2 index terminal '–offrate' option. Default value is "4"

Hisat2.Alignment.run
                  Whether to run 'HISAT2 alignment' step in this function step. Default value is
                  TRUE. Set FALSE to skip 'HISAT2 alignment' step.

Hisat2.Alignment.num.parallel.threads
                 Specify the number of processing threads (CPUs) to use for Hisat2 alignment
                 step. The default is ″1″
Hisat2.Alignment.skip
                 Hisat2 alignment terminal '-s/–skip' option. Default value is ″0″
Hisat2.Alignment.trim5
                 Hisat2 alignment terminal '-5/–trim5' option. Default value is ″0″
Hisat2.Alignment.trim3
                 Hisat2 alignment terminal '-3/–trim3' option. Default value is ″0″
Hisat2.Alignment.n.ceil.1.function.type
                 Hisat2 alignment terminal '–n-ceil' option. Default value is ″L″
Hisat2.Alignment.n.ceil.2.constant.term
                 Hisat2 alignment terminal '–n-ceil' option. Default value is ″0″
Hisat2.Alignment.n.ceil.3.coefficient
                 Hisat2 alignment terminal '–n-ceil' option. Default value is ″0.15″
Hisat2.Alignment.mp.MX
                 Hisat2 alignment terminal '–mp MX' option. Default value is ″6″
Hisat2.Alignment.mp.MN
                 Hisat2 alignment terminal '–mp MN' option. Default value is ″2″
Hisat2.Alignment.sp.MX
                 Hisat2 alignment terminal '–sp MX' option. Default value is ″2″
Hisat2.Alignment.sp.MN
                 Hisat2 alignment terminal '–sp MN' option. Default value is ″1″
Hisat2.Alignment.np
                 Hisat2 alignment terminal '–np' option. Default value is ″1″
Hisat2.Alignment.rdg.1
                 Hisat2 alignment terminal '–rdg' first option. Default value is ″5″
Hisat2.Alignment.rdg.2
                 Hisat2 alignment terminal '–rdg' first option. Default value is ″3″
Hisat2.Alignment.rfg.1
                 Hisat2 alignment terminal '–rfg' first option. Default value is ″5″
Hisat2.Alignment.rfg.2
                 Hisat2 alignment terminal '–rfg' first option. Default value is ″3″
Hisat2.Alignment.score.min.1.function.type
                 Hisat2 alignment terminal '–rdg' first option. Default value is ″L″
Hisat2.Alignment.score.min.2.constant.term
                 Hisat2 alignment terminal '–rdg' first option. Default value is ″0″
Hisat2.Alignment.score.min.3.coefficient
                 Hisat2 alignment terminal '–rdg' first option. Default value is ″-0.2″
Hisat2.Alignment.pen.cansplice
                 Hisat2 alignment terminal '–pen-cansplice' first option. Default value is ″-0″
Hisat2.Alignment.penc.noncansplice
                 Hisat2 alignment terminal '–pen-noncansplice' option. Default value is ″12″
Hisat2.Alignment.pen.canintronlen.1.function.type
                 Hisat2 alignment terminal '–pen-canintronlen' first option. Default value is ″G″

Hisat2.Alignment.pen.canintronlen.2.constant.term

Hisat2 alignment terminal '–pen-canintronlen' second option. Default value is
"-8"

Hisat2.Alignment.pen.canintronlen.3.coefficient

Hisat2 alignment terminal '–pen-canintronlen' third option. Default value is "1"

Hisat2.Alignment.pen.noncanintronlen.1.function.type

Hisat2 alignment terminal '–pen-noncanintronlen' first option. Default value is
"G"

Hisat2.Alignment.pen.noncanintronlen.2.constant.term

Hisat2 alignment terminal '–pen-noncanintronlen' second option. Default value
is "-8"

Hisat2.Alignment.pen.noncanintronlen.3.coefficient

Hisat2 alignment terminal '–pen-noncanintronlen' third option. Default value is
"1"

Hisat2.Alignment.min.intronlen

Hisat2 alignment terminal '–min-intronlen' option. Default value is "20"

Hisat2.Alignment.max.intronlen

Hisat2 alignment terminal '–max-intronlen' option. Default value is "20"

Hisat2.Alignment.rna.strandness

Hisat2 alignment terminal '–rna-strandness' option. Default value is "None"

Hisat2.Alignment.k

Hisat2 alignment terminal '-k' option. Default value is "5"

Hisat2.Alignment.max.seeds

Hisat2 alignment terminal '–max-seeds' option. Default value is "5"

Hisat2.Alignment.secondary

Hisat2 alignment terminal '–secondary' option. Default value is "FALSE"

Hisat2.Alignment.minins

Hisat2 alignment terminal '-I/–minins' option. Default value is "0"

Hisat2.Alignment.maxins

Hisat2 alignment terminal '-X/–maxins' option. Default value is "500"

Hisat2.Alignment.seed

Hisat2 alignment terminal '-X/–maxins' option. Default value is "0"

STAR.Index.num.parallel.threads

Specify the number of processing threads (CPUs) to use for STAR index step.
The default is "1"

STAR.Index.sjdbOverhang.Read.length

STAR index terminal '–sjdbOverhang' option. Default value is "100"

STAR.Index.genomeSAindexNbases

STAR index terminal '–genomeSAindexNbases' option. Default value is "14"

STAR.Index.genomeChrBinNbits

STAR index terminal '–genomeChrBinNbits' option. Default value is "18"

STAR.Index.genomeSAsparseD

STAR index terminal '–genomeSAsparseD' option. Default value is "1"

STAR.Alignment.run

Whether to run 'STAR index' step in this function step. Default value is FALSE.
Set TRUE to run STAR alignment step. (need to set Hisat2.Index.run to FALSE)

STAR.Alignment.num.parallel.threads
          Specify the number of processing threads (CPUs) to use for STAR alignment
          step. The default is "1"
STAR.Alignment.genomeLoad
          STAR alignment terminal '–genomeLoad' option. Default value is "NoSharedMemory"
STAR.Alignment.readMapNumber
          STAR alignment terminal '–readMapNumber' option. Default value is "-1"
STAR.Alignment.clip3pNbases
          STAR alignment terminal '–clip3pNbases' option. Default value is "0"
STAR.Alignment.clip5pNbases
          STAR alignment terminal '–clip5pNbases' option. Default value is "0"
STAR.Alignment.clip3pAdapterSeq
          STAR alignment terminal '–clip3pAdapterSeq' option. Default value is "-"
STAR.Alignment.clip3pAdapterMMp
          STAR alignment terminal '–clip3pAdapterMMp' option. Default value is "0.1"
STAR.Alignment.clip3pAfterAdapterNbases
          STAR alignment terminal '–clip3pAfterAdapterNbases' option. Default value
          is "0"
STAR.Alignment.limitGenomeGenerateRAM
          STAR alignment terminal '–limitGenomeGenerateRAM' option. Default value
          is "31000000000"
STAR.Alignment.limitIObufferSize
          STAR alignment terminal '–limitIObufferSize' option. Default value is "150000000"
STAR.Alignment.limitOutSAMoneReadBytes
          STAR alignment terminal '–limitOutSAMoneReadBytes' option. Default value
          is "100000"
STAR.Alignment.limitOutSJoneRead
          STAR alignment terminal '–limitOutSJoneRead' option. Default value is "1000"
STAR.Alignment.limitOutSJcollapsed
          STAR alignment terminal '–limitOutSJcollapsed' option. Default value is "1000000"
STAR.Alignment.limitBAMsortRAM
          STAR alignment terminal '–limitBAMsortRAM' option. Default value is "0"
STAR.Alignment.outReadsUnmapped
          STAR alignment terminal '–outReadsUnmapped' option. Default value is "None"
STAR.Alignment.outQSconversionAdd
          STAR alignment terminal '–outQSconversionAdd' option. Default value is "0"
STAR.Alignment.outSAMprimaryFlag
          STAR alignment terminal '–outSAMprimaryFlag' option. Default value is "OneBestScore"
STAR.Alignment.outSAMmapqUnique
          STAR alignment terminal '–outSAMmapqUnique' option. Default value is "255"
STAR.Alignment.scoreGap
          STAR alignment terminal '–scoreGap' option. Default value is "0"
STAR.Alignment.scoreGapNoncan
          STAR alignment terminal '–scoreGapNoncan' option. Default value is "-8"
STAR.Alignment.scoreGapGCAG
          STAR alignment terminal '–scoreGapGCAG' option. Default value is "-4"

STAR.Alignment.scoreGapATAC

        STAR alignment terminal '–scoreGapATAC' option. Default value is "-8"

STAR.Alignment.scoreGenomicLengthLog2scale

        STAR alignment terminal '–scoreGenomicLengthLog2scale' option. Default value is "-0.25"

STAR.Alignment.scoreDelOpen

        STAR alignment terminal '–scoreDelOpen' option. Default value is "-2"

STAR.Alignment.scoreDelBase

        STAR alignment terminal '–scoreDelBase' option. Default value is "-2"

STAR.Alignment.scoreInsOpen

        STAR alignment terminal '–scoreInsOpen' option. Default value is "-2"

STAR.Alignment.scoreInsBase

        STAR alignment terminal '–scoreInsBase' option. Default value is "-2"

STAR.Alignment.scoreStitchSJshift

        STAR alignment terminal '–scoreStitchSJshift' option. Default value is "1"

STAR.Alignment.seedSearchStartLmax

        STAR alignment terminal '–scoreStitchSJshift' option. Default value is "50"

STAR.Alignment.seedSearchStartLmaxOverLread

        STAR alignment terminal '–seedSearchStartLmaxOverLread' option. Default value is "1.0"

STAR.Alignment.seedSearchLmax

        STAR alignment terminal '–seedSearchLmax' option. Default value is "0"

STAR.Alignment.seedMultimapNmax

        STAR alignment terminal '–seedMultimapNmax' option. Default value is "10000"

STAR.Alignment.seedPerReadNmax

        STAR alignment terminal '–seedPerReadNmax' option. Default value is "1000"

STAR.Alignment.seedPerWindowNmax

        STAR alignment terminal '–seedPerWindowNmax' option. Default value is "50"

STAR.Alignment.seedNoneLociPerWindow

        STAR alignment terminal '–seedNoneLociPerWindow' option. Default value is "10"

STAR.Alignment.alignIntronMin

        STAR alignment terminal '–alignIntronMin' option. Default value is "21"

STAR.Alignment.alignIntronMax

        STAR alignment terminal '–alignIntronMax' option. Default value is "0"

STAR.Alignment.alignMatesGapMax

        STAR alignment terminal '–alignMatesGapMax' option. Default value is "0"

STAR.Alignment.alignSJoverhangMin

        STAR alignment terminal '–alignSJoverhangMin' option. Default value is "5"

STAR.Alignment.alignSJDBoverhangMin

        STAR alignment terminal '–alignSJDBoverhangMin' option. Default value is "3"

STAR.Alignment.alignSplicedMateMapLmin

        STAR alignment terminal '–alignSplicedMateMapLmin' option. Default value is "0"

STAR.Alignment.alignSplicedMateMapLminOverLmate

> STAR alignment terminal '–alignSplicedMateMapLminOverLmate' option. Default value is `"0.66"`

STAR.Alignment.alignWindowsPerReadNmax

> STAR alignment terminal '–alignWindowsPerReadNmax' option. Default value is `"10000"`

STAR.Alignment.alignTranscriptsPerWindowNmax

> STAR alignment terminal '–alignTranscriptsPerWindowNmax' option. Default value is `"100"`

STAR.Alignment.alignTranscriptsPerReadNmax

> STAR alignment terminal '–alignTranscriptsPerReadNmax' option. Default value is `"10000"`

STAR.Alignment.alignEndsType

> STAR alignment terminal '–alignEndsType' option. Default value is `"Local"`

STAR.Alignment.winAnchorMultimapNmax

> STAR alignment terminal '–winAnchorMultimapNmax' option. Default value is `"50"`

STAR.Alignment.winBinNbits

> STAR alignment terminal '–winBinNbits' option. Default value is `"16"`

STAR.Alignment.winAnchorDistNbins

> STAR alignment terminal '–winAnchorDistNbins' option. Default value is `"9"`

STAR.Alignment.winFlankNbins

> STAR alignment terminal '–winFlankNbins' option. Default value is `"4"`

Rsamtools.Bam.run

> Whether to run 'Rsamtools SAM to BAM' step in this function step. Default value is TRUE. Set FALSE to skip 'Rsamtools SAM to BAM' step.

Samtools.Bam.num.parallel.threads

> Specify the number of processing threads (CPUs) to use for Samtools sam to bam step. The default is `"1"`

Rsamtools.nCores

> The number of cores to use when running 'Rsamtools' step. Default value is 1

StringTie.Assemble.run

> Whether to run 'StringTie assembly' step in this function step. Default value is TRUE. Set FALSE to skip 'StringTie assembly' step.

Stringtie.Assembly.num.parallel.threads

> Specify the number of processing threads (CPUs) to use for Stringtie assembly. The default is `"1"`

Stringtie.Assembly.f

> Stringtie assembly terminal '-f' option. Default value is `"0.1"`

Stringtie.Assembly.m

> Stringtie assembly terminal '-m' option. Default value is `"200"`

Stringtie.Assembly.c

> Stringtie assembly terminal '-c' option. Default value is `"2.5"`

Stringtie.Assembly.g

> Stringtie assembly terminal '-g' option. Default value is `"50"`

Stringtie.Assembly.M

> Stringtie assembly terminal '-M' option. Default value is `"0.95"`

StringTie.Merge.Trans.run
>   Whether to run 'StringTie GTF merging' step in this function step. Default value
>   is TRUE. Set FALSE to skip 'StringTie GTF merging' step.

Stringtie.Merge.num.parallel.threads
>   Specify the number of processing threads (CPUs) to use for Stringtie merge step.
>   The default is "1"

Gffcompare.Ref.Sample.run
>   Whether to run 'Gffcompare comparison' step in this function step. Default
>   value is TRUE. Set FALSE to skip 'Gffcompare comparison' step.

StringTie.Ballgown.run
>   Whether to run 'StringTie ballgown creation' step in this function step. Default
>   value is TRUE. Set FALSE to skip 'StringTie ballgown creation' step.

Stringtie.2.Ballgown.num.parallel.threads
>   Specify the number of processing threads (CPUs) to use for Stringtie to ball-
>   gown step. The default is "1"

PreDECountTable.run
>   Whether to run 'gene raw reads count creation' step in this function step. Default
>   value is TRUE. Set FALSE to skip 'gene raw reads count creation' step.

check.s4.print Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in
'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking RNASeqRParam
will not be in 'Rscript_out/Environment_Set.Rout'.

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
## Before run this function, make sure \code{RNASeqEnvironmentSet_CMD()}
##(or\code{RNASeqEnvironmentSet()}) is executed successfully.
RNASeqReadProcess(RNASeqRParam       = yeast,
                  num.parallel.threads = 10)
## End(Not run)
```

RNASeqReadProcess_CMD *RNASeqReadProcess_CMD*

Description

Process raw reads for RNA-Seq workflow in background.
This function do 5 things :

1. 'Hisat2' : aligns raw reads to reference genome. If `indices.optional` in RNASeqRParam is FALSE, Hisat2 indices will be created.

2. 'Rsamtools': converts '.sam' files to '.bam' files.

3. 'Stringtie': assembles alignments into transcript.

4. 'Gffcompare': examines how transcripts compare with the reference annotation.

5. 'Stringtie': creates input files for ballgown, edgeR and DESeq2.

6. raw reads count: create raw reads count for DESeq2 and edgeR

Before running this function, `RNASeqEnvironmentSet_CMD()` or `RNASeqEnvironmentSet()` must be executed successfully.
If you want to process raw reads for the following RNA-Seq workflow in R shell, please see `RNASeqReadProcess()` function.

Usage

```
RNASeqReadProcess_CMD(RNASeqRParam, SAMtools.or.Rsamtools = "Rsamtools",
  Hisat2.Index.run = TRUE, Hisat2.Index.num.parallel.threads = "1",
  Hisat2.Index.large.index = FALSE,
  Hisat2.Index.local.ftab.chars = "6",
  Hisat2.Index.local.off.rate = "3", Hisat2.Index.ftab.chars = "10",
  Hisat2.Index.off.rate = "4", Hisat2.Alignment.run = TRUE,
  Hisat2.Alignment.num.parallel.threads = "1",
  Hisat2.Alignment.skip = "0", Hisat2.Alignment.trim5 = "0",
  Hisat2.Alignment.trim3 = "0",
  Hisat2.Alignment.n.ceil.1.function.type = "L",
  Hisat2.Alignment.n.ceil.2.constant.term = "0",
  Hisat2.Alignment.n.ceil.3.coefficient = "0.15",
  Hisat2.Alignment.mp.MX = "6", Hisat2.Alignment.mp.MN = "2",
  Hisat2.Alignment.sp.MX = "2", Hisat2.Alignment.sp.MN = "1",
  Hisat2.Alignment.np = "1", Hisat2.Alignment.rdg.1 = "5",
  Hisat2.Alignment.rdg.2 = "3", Hisat2.Alignment.rfg.1 = "5",
  Hisat2.Alignment.rfg.2 = "3",
  Hisat2.Alignment.score.min.1.function.type = "L",
  Hisat2.Alignment.score.min.2.constant.term = "0",
  Hisat2.Alignment.score.min.3.coefficient = "-0.2",
```

```
Hisat2.Alignment.pen.cansplice = "0",
Hisat2.Alignment.penc.noncansplice = "12",
Hisat2.Alignment.pen.canintronlen.1.function.type = "G",
Hisat2.Alignment.pen.canintronlen.2.constant.term = "-8",
Hisat2.Alignment.pen.canintronlen.3.coefficient = "1",
Hisat2.Alignment.pen.noncanintronlen.1.function.type = "G",
Hisat2.Alignment.pen.noncanintronlen.2.constant.term = "-8",
Hisat2.Alignment.pen.noncanintronlen.3.coefficient = "1",
Hisat2.Alignment.min.intronlen = "20",
Hisat2.Alignment.max.intronlen = "500000",
Hisat2.Alignment.rna.strandness = "None", Hisat2.Alignment.k = "5",
Hisat2.Alignment.max.seeds = "5", Hisat2.Alignment.secondary = FALSE,
Hisat2.Alignment.minins = "0", Hisat2.Alignment.maxins = "500",
Hisat2.Alignment.seed = "0", STAR.Index.num.parallel.threads = "1",
STAR.Index.sjdbOverhang.Read.length = "100",
STAR.Index.genomeSAindexNbases = "14",
STAR.Index.genomeChrBinNbits = "18",
STAR.Index.genomeSAsparseD = "1", STAR.Alignment.run = FALSE,
STAR.Alignment.num.parallel.threads = "1",
STAR.Alignment.genomeLoad = "NoSharedMemory",
STAR.Alignment.readMapNumber = "-1",
STAR.Alignment.clip3pNbases = "0", STAR.Alignment.clip5pNbases = "0",
STAR.Alignment.clip3pAdapterSeq = "-",
STAR.Alignment.clip3pAdapterMMp = "0.1",
STAR.Alignment.clip3pAfterAdapterNbases = "0",
STAR.Alignment.limitGenomeGenerateRAM = "31000000000",
STAR.Alignment.limitIObufferSize = "150000000",
STAR.Alignment.limitOutSAMoneReadBytes = "100000",
STAR.Alignment.limitOutSJoneRead = "1000",
STAR.Alignment.limitOutSJcollapsed = "1000000",
STAR.Alignment.limitBAMsortRAM = "0",
STAR.Alignment.outReadsUnmapped = "None",
STAR.Alignment.outQSconversionAdd = "0",
STAR.Alignment.outSAMprimaryFlag = "OneBestScore",
STAR.Alignment.outSAMmapqUnique = "255",
STAR.Alignment.scoreGap = "0", STAR.Alignment.scoreGapNoncan = "-8",
STAR.Alignment.scoreGapGCAG = "-4",
STAR.Alignment.scoreGapATAC = "-8",
STAR.Alignment.scoreGenomicLengthLog2scale = "-0.25",
STAR.Alignment.scoreDelOpen = "-2",
STAR.Alignment.scoreDelBase = "-2",
STAR.Alignment.scoreInsOpen = "-2",
STAR.Alignment.scoreInsBase = "-2",
STAR.Alignment.scoreStitchSJshift = "1",
STAR.Alignment.seedSearchStartLmax = "50",
STAR.Alignment.seedSearchStartLmaxOverLread = "1.0",
STAR.Alignment.seedSearchLmax = "0",
STAR.Alignment.seedMultimapNmax = "10000",
```

```
STAR.Alignment.seedPerReadNmax = "1000",
STAR.Alignment.seedPerWindowNmax = "50",
STAR.Alignment.seedNoneLociPerWindow = "10",
STAR.Alignment.alignIntronMin = "21",
STAR.Alignment.alignIntronMax = "0",
STAR.Alignment.alignMatesGapMax = "0",
STAR.Alignment.alignSJoverhangMin = "5",
STAR.Alignment.alignSJDBoverhangMin = "3",
STAR.Alignment.alignSplicedMateMapLmin = "0",
STAR.Alignment.alignSplicedMateMapLminOverLmate = "0.66",
STAR.Alignment.alignWindowsPerReadNmax = "10000",
STAR.Alignment.alignTranscriptsPerWindowNmax = "100",
STAR.Alignment.alignTranscriptsPerReadNmax = "10000",
STAR.Alignment.alignEndsType = "Local",
STAR.Alignment.winAnchorMultimapNmax = "50",
STAR.Alignment.winBinNbits = "16",
STAR.Alignment.winAnchorDistNbins = "9",
STAR.Alignment.winFlankNbins = "4", Rsamtools.Bam.run = TRUE,
Samtools.Bam.num.parallel.threads = "1", Rsamtools.nCores = "1",
StringTie.Assemble.run = TRUE,
Stringtie.Assembly.num.parallel.threads = "1",
Stringtie.Assembly.f = "0.1", Stringtie.Assembly.m = "200",
Stringtie.Assembly.c = "2.5", Stringtie.Assembly.g = "50",
Stringtie.Assembly.M = "0.95", StringTie.Merge.Trans.run = TRUE,
Stringtie.Merge.num.parallel.threads = "1",
Gffcompare.Ref.Sample.run = TRUE, StringTie.Ballgown.run = TRUE,
Stringtie.2.Ballgown.num.parallel.threads = "1",
PreDECountTable.run = TRUE, run = TRUE, check.s4.print = TRUE)
```

Arguments

RNASeqRParam   S4 object instance of experiment-related parameters

SAMtools.or.Rsamtools

Default value is Rsamtools. User can set to SAMtools to use command-line-based 'samtools' instead.

Hisat2.Index.run

Whether to run 'HISAT2 index' step in this function step. Default value is TRUE. Set FALSE to skip 'HISAT2 index' step.

Hisat2.Index.num.parallel.threads

Specify the number of processing threads (CPUs) to use for Hisat2 index step. The default is "1"

Hisat2.Index.large.index

Hisat2 index terminal '–large-index' option. Default value is FALSE

Hisat2.Index.local.ftab.chars

Hisat2 index terminal '-t/–ftabchars' option. Default value is "6"

Hisat2.Index.local.off.rate

Hisat2 index terminal '–localoffrate' option. Default value is "3"

Hisat2.Index.ftab.chars

> Hisat2 index terminal '–localftabchars' option. Default value is "10"

Hisat2.Index.off.rate

> Hisat2 index terminal '–offrate' option. Default value is "4"

Hisat2.Alignment.run

> Whether to run 'HISAT2 alignment' step in this function step. Default value is TRUE. Set FALSE to skip 'HISAT2 alignment' step.

Hisat2.Alignment.num.parallel.threads

> Specify the number of processing threads (CPUs) to use for Hisat2 alignment step. The default is "1"

Hisat2.Alignment.skip

> Hisat2 alignment terminal '-s/–skip' option. Default value is "0"

Hisat2.Alignment.trim5

> Hisat2 alignment terminal '-5/–trim5' option. Default value is "0"

Hisat2.Alignment.trim3

> Hisat2 alignment terminal '-3/–trim3' option. Default value is "0"

Hisat2.Alignment.n.ceil.1.function.type

> Hisat2 alignment terminal '–n-ceil' option. Default value is "L"

Hisat2.Alignment.n.ceil.2.constant.term

> Hisat2 alignment terminal '–n-ceil' option. Default value is "0"

Hisat2.Alignment.n.ceil.3.coefficient

> Hisat2 alignment terminal '–n-ceil' option. Default value is "0.15"

Hisat2.Alignment.mp.MX

> Hisat2 alignment terminal '–mp MX' option. Default value is "6"

Hisat2.Alignment.mp.MN

> Hisat2 alignment terminal '–mp MN' option. Default value is "2"

Hisat2.Alignment.sp.MX

> Hisat2 alignment terminal '–sp MX' option. Default value is "2"

Hisat2.Alignment.sp.MN

> Hisat2 alignment terminal '–sp MN' option. Default value is "1"

Hisat2.Alignment.np

> Hisat2 alignment terminal '–np' option. Default value is "1"

Hisat2.Alignment.rdg.1

> Hisat2 alignment terminal '–rdg' first option. Default value is "5"

Hisat2.Alignment.rdg.2

> Hisat2 alignment terminal '–rdg' first option. Default value is "3"

Hisat2.Alignment.rfg.1

> Hisat2 alignment terminal '–rfg' first option. Default value is "5"

Hisat2.Alignment.rfg.2

> Hisat2 alignment terminal '–rfg' first option. Default value is "3"

Hisat2.Alignment.score.min.1.function.type

> Hisat2 alignment terminal '–rdg' first option. Default value is "L"

Hisat2.Alignment.score.min.2.constant.term

> Hisat2 alignment terminal '–rdg' first option. Default value is "0"

Hisat2.Alignment.score.min.3.coefficient
Hisat2 alignment terminal '–rdg' first option. Default value is "-0.2"

Hisat2.Alignment.pen.cansplice
Hisat2 alignment terminal '–pen-cansplice' first option. Default value is "-0"

Hisat2.Alignment.penc.noncansplice
Hisat2 alignment terminal '–pen-noncansplice' option. Default value is "12"

Hisat2.Alignment.pen.canintronlen.1.function.type
Hisat2 alignment terminal '–pen-canintronlen' first option. Default value is "G"

Hisat2.Alignment.pen.canintronlen.2.constant.term
Hisat2 alignment terminal '–pen-canintronlen' second option. Default value is
"-8"

Hisat2.Alignment.pen.canintronlen.3.coefficient
Hisat2 alignment terminal '–pen-canintronlen' third option. Default value is "1"

Hisat2.Alignment.pen.noncanintronlen.1.function.type
Hisat2 alignment terminal '–pen-noncanintronlen' first option. Default value is
"G"

Hisat2.Alignment.pen.noncanintronlen.2.constant.term
Hisat2 alignment terminal '–pen-noncanintronlen' second option. Default value
is "-8"

Hisat2.Alignment.pen.noncanintronlen.3.coefficient
Hisat2 alignment terminal '–pen-noncanintronlen' third option. Default value is
"1"

Hisat2.Alignment.min.intronlen
Hisat2 alignment terminal '–min-intronlen' option. Default value is "20"

Hisat2.Alignment.max.intronlen
Hisat2 alignment terminal '–max-intronlen' option. Default value is "20"

Hisat2.Alignment.rna.strandness
Hisat2 alignment terminal '–rna-strandness' option. Default value is "None"

Hisat2.Alignment.k
Hisat2 alignment terminal '-k' option. Default value is "5"

Hisat2.Alignment.max.seeds
Hisat2 alignment terminal '–max-seeds' option. Default value is "5"

Hisat2.Alignment.secondary
Hisat2 alignment terminal '–secondary' option. Default value is "FALSE"

Hisat2.Alignment.minins
Hisat2 alignment terminal '-I/–minins' option. Default value is "0"

Hisat2.Alignment.maxins
Hisat2 alignment terminal '-X/–maxins' option. Default value is "500"

Hisat2.Alignment.seed
Hisat2 alignment terminal '-X/–maxins' option. Default value is "0"

STAR.Index.num.parallel.threads
Specify the number of processing threads (CPUs) to use for STAR index step.
The default is "1"

STAR.Index.sjdbOverhang.Read.length
STAR index terminal '–sjdbOverhang' option. Default value is "100"

STAR.Index.genomeSAindexNbases

> STAR index terminal '–genomeSAindexNbases' option. Default value is "14"

STAR.Index.genomeChrBinNbits

> STAR index terminal '–genomeChrBinNbits' option. Default value is "18"

STAR.Index.genomeSAsparseD

> STAR index terminal '–genomeSAsparseD' option. Default value is "1"

STAR.Alignment.run

> Whether to run 'STAR index' step in this function step. Default value is FALSE.
> Set TRUE to run STAR alignment step. (need to set Hisat2.Index.run to FALSE)

STAR.Alignment.num.parallel.threads

> Specify the number of processing threads (CPUs) to use for STAR alignment
> step. The default is "1"

STAR.Alignment.genomeLoad

> STAR alignment terminal '–genomeLoad' option. Default value is "NoSharedMemory"

STAR.Alignment.readMapNumber

> STAR alignment terminal '–readMapNumber' option. Default value is "-1"

STAR.Alignment.clip3pNbases

> STAR alignment terminal '–clip3pNbases' option. Default value is "0"

STAR.Alignment.clip5pNbases

> STAR alignment terminal '–clip5pNbases' option. Default value is "0"

STAR.Alignment.clip3pAdapterSeq

> STAR alignment terminal '–clip3pAdapterSeq' option. Default value is "-"

STAR.Alignment.clip3pAdapterMMp

> STAR alignment terminal '–clip3pAdapterMMp' option. Default value is "0.1"

STAR.Alignment.clip3pAfterAdapterNbases

> STAR alignment terminal '–clip3pAfterAdapterNbases' option. Default value
> is "0"

STAR.Alignment.limitGenomeGenerateRAM

> STAR alignment terminal '–limitGenomeGenerateRAM' option. Default value
> is "31000000000"

STAR.Alignment.limitIObufferSize

> STAR alignment terminal '–limitIObufferSize' option. Default value is "150000000"

STAR.Alignment.limitOutSAMoneReadBytes

> STAR alignment terminal '–limitOutSAMoneReadBytes' option. Default value
> is "100000"

STAR.Alignment.limitOutSJoneRead

> STAR alignment terminal '–limitOutSJoneRead' option. Default value is "1000"

STAR.Alignment.limitOutSJcollapsed

> STAR alignment terminal '–limitOutSJcollapsed' option. Default value is "1000000"

STAR.Alignment.limitBAMsortRAM

> STAR alignment terminal '–limitBAMsortRAM' option. Default value is "0"

STAR.Alignment.outReadsUnmapped

> STAR alignment terminal '–outReadsUnmapped' option. Default value is "None"

STAR.Alignment.outQSconversionAdd

> STAR alignment terminal '–outQSconversionAdd' option. Default value is "0"

STAR.Alignment.outSAMprimaryFlag
                STAR alignment terminal '–outSAMprimaryFlag' option. Default value is "OneBestScore"

STAR.Alignment.outSAMmapqUnique
                STAR alignment terminal '–outSAMmapqUnique' option. Default value is "255"

STAR.Alignment.scoreGap
                STAR alignment terminal '–scoreGap' option. Default value is "0"

STAR.Alignment.scoreGapNoncan
                STAR alignment terminal '–scoreGapNoncan' option. Default value is "-8"

STAR.Alignment.scoreGapGCAG
                STAR alignment terminal '–scoreGapGCAG' option. Default value is "-4"

STAR.Alignment.scoreGapATAC
                STAR alignment terminal '–scoreGapATAC' option. Default value is "-8"

STAR.Alignment.scoreGenomicLengthLog2scale
                STAR alignment terminal '–scoreGenomicLengthLog2scale' option. Default
                value is "-0.25"

STAR.Alignment.scoreDelOpen
                STAR alignment terminal '–scoreDelOpen' option. Default value is "-2"

STAR.Alignment.scoreDelBase
                STAR alignment terminal '–scoreDelBase' option. Default value is "-2"

STAR.Alignment.scoreInsOpen
                STAR alignment terminal '–scoreInsOpen' option. Default value is "-2"

STAR.Alignment.scoreInsBase
                STAR alignment terminal '–scoreInsBase' option. Default value is "-2"

STAR.Alignment.scoreStitchSJshift
                STAR alignment terminal '–scoreStitchSJshift' option. Default value is "1"

STAR.Alignment.seedSearchStartLmax
                STAR alignment terminal '–scoreStitchSJshift' option. Default value is "50"

STAR.Alignment.seedSearchStartLmaxOverLread
                STAR alignment terminal '–seedSearchStartLmaxOverLread' option. Default
                value is "1.0"

STAR.Alignment.seedSearchLmax
                STAR alignment terminal '–seedSearchLmax' option. Default value is "0"

STAR.Alignment.seedMultimapNmax
                STAR alignment terminal '–seedMultimapNmax' option. Default value is "10000"

STAR.Alignment.seedPerReadNmax
                STAR alignment terminal '–seedPerReadNmax' option. Default value is "1000"

STAR.Alignment.seedPerWindowNmax
                STAR alignment terminal '–seedPerWindowNmax' option. Default value is
                "50"

STAR.Alignment.seedNoneLociPerWindow
                STAR alignment terminal '–seedNoneLociPerWindow' option. Default value is
                "10"

STAR.Alignment.alignIntronMin
                STAR alignment terminal '–alignIntronMin' option. Default value is "21"

STAR.Alignment.alignIntronMax
                STAR alignment terminal '–alignIntronMax' option. Default value is "0"

STAR.Alignment.alignMatesGapMax
> STAR alignment terminal '–alignMatesGapMax' option. Default value is "0"

STAR.Alignment.alignSJoverhangMin
> STAR alignment terminal '–alignSJoverhangMin' option. Default value is "5"

STAR.Alignment.alignSJDBoverhangMin
> STAR alignment terminal '–alignSJDBoverhangMin' option. Default value is "3"

STAR.Alignment.alignSplicedMateMapLmin
> STAR alignment terminal '–alignSplicedMateMapLmin' option. Default value is "0"

STAR.Alignment.alignSplicedMateMapLminOverLmate
> STAR alignment terminal '–alignSplicedMateMapLminOverLmate' option. Default value is "0.66"

STAR.Alignment.alignWindowsPerReadNmax
> STAR alignment terminal '–alignWindowsPerReadNmax' option. Default value is "10000"

STAR.Alignment.alignTranscriptsPerWindowNmax
> STAR alignment terminal '–alignTranscriptsPerWindowNmax' option. Default value is "100"

STAR.Alignment.alignTranscriptsPerReadNmax
> STAR alignment terminal '–alignTranscriptsPerReadNmax' option. Default value is "10000"

STAR.Alignment.alignEndsType
> STAR alignment terminal '–alignEndsType' option. Default value is "Local"

STAR.Alignment.winAnchorMultimapNmax
> STAR alignment terminal '–winAnchorMultimapNmax' option. Default value is "50"

STAR.Alignment.winBinNbits
> STAR alignment terminal '–winBinNbits' option. Default value is "16"

STAR.Alignment.winAnchorDistNbins
> STAR alignment terminal '–winAnchorDistNbins' option. Default value is "9"

STAR.Alignment.winFlankNbins
> STAR alignment terminal '–winFlankNbins' option. Default value is "4"

Rsamtools.Bam.run
> Whether to run 'Rsamtools SAM to BAM' step in this function step. Default value is TRUE. Set FALSE to skip 'Rsamtools SAM to BAM' step.

Samtools.Bam.num.parallel.threads
> Specify the number of processing threads (CPUs) to use for Samtools sam to bam step. The default is "1"

Rsamtools.nCores
> The number of cores to use when running 'Rsamtools' step. Default value is 1

StringTie.Assemble.run
> Whether to run 'StringTie assembly' step in this function step. Default value is TRUE. Set FALSE to skip 'StringTie assembly' step.

Stringtie.Assembly.num.parallel.threads
> Specify the number of processing threads (CPUs) to use for Stringtie assembly. The default is "1"

Stringtie.Assembly.f
               Stringtie assembly terminal '-f' option. Default value is "0.1"

Stringtie.Assembly.m
               Stringtie assembly terminal '-m' option. Default value is "200"

Stringtie.Assembly.c
               Stringtie assembly terminal '-c' option. Default value is "2.5"

Stringtie.Assembly.g
               Stringtie assembly terminal '-g' option. Default value is "50"

Stringtie.Assembly.M
               Stringtie assembly terminal '-M' option. Default value is "0.95"

StringTie.Merge.Trans.run
               Whether to run 'StringTie GTF merging' step in this function step. Default value
               is TRUE. Set FALSE to skip 'StringTie GTF merging' step.

Stringtie.Merge.num.parallel.threads
               Specify the number of processing threads (CPUs) to use for Stringtie merge step.
               The default is "1"

Gffcompare.Ref.Sample.run
               Whether to run 'Gffcompare comparison' step in this function step. Default
               value is TRUE. Set FALSE to skip 'Gffcompare comparison' step.

StringTie.Ballgown.run
               Whether to run 'StringTie ballgown creation' step in this function step. Default
               value is TRUE. Set FALSE to skip 'StringTie ballgown creation' step.

Stringtie.2.Ballgown.num.parallel.threads
               Specify the number of processing threads (CPUs) to use for Stringtie to ball-
               gown step. The default is "1"

PreDECountTable.run
               Whether to run 'gene raw reads count creation' step in this function step. Default
               value is TRUE. Set FALSE to skip 'gene raw reads count creation' step.

run   Default value is TRUE. If TRUE, 'Rscript/Environment_Set.R' will be created and
               executed. The output log will be stored in 'Rscript_out/Environment_Set.Rout'.
               If False, 'Rscript/Environment_Set.R' will be created without executed.

check.s4.print Default TRUE. If TRUE, the result of checking RNASeqRParam will be reported in
               'Rscript_out/Environment_Set.Rout'. If FALSE, the result of checking RNASeqRParam
               will not be in 'Rscript_out/Environment_Set.Rout'.

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
## Before run this function, make sure \code{RNASeqEnvironmentSet_CMD()}
## (or\code{RNASeqEnvironmentSet()}) is executed successfully.
RNASeqReadProcess_CMD(RNASeqRParam = yeast,
                      num.parallel.threads = 10)
## End(Not run)
```

RNASeqRParam-class   *RNASeqR*

Description

An S4 class for checking and storing RNA-Seq workflow parameters of this package.

Slots

os.type 'linux' or 'osx'. The operating system type.

python.variable A list storing python environment. (check.answer,python.version)

python.2to3 Logical value whether 2to3 command is available on the workstation.

path.prefix Path prefix of 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/' and 'Rscript_out/' directories.

input.path.prefix Path prefix of 'input_files/' directory,

genome.name Variable of genome name defined in this RNA-Seq workflow (ex. genome.name.fa, genome.name.gtf).

sample.pattern Regular expression of paired-end fastq.gz files under 'input_files/raw_fastq.gz'. Expression not includes _[1,2].fastq.gz.

independent.variable Independent variable for the biological. experiment design of two-group RNA-Seq workflow.

case.group Group name of the case group.

control.group Group name of the control group.

indices.optional Logical value whether 'indices/' is exit in 'input_files/'.

fastq.gz.type Specify the fastq.gz file type. 'PE' represents paired-end and 'SE' represents single-end.

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
"@"(yeast, os.type)
"@"(yeast, python.variable)
"@"(yeast, python.2to3)
"@"(yeast, path.prefix)
"@"(yeast, input.path.prefix)
"@"(yeast, genome.name)
"@"(yeast, sample.pattern)
"@"(yeast, independent.variable)
"@"(yeast, case.group)
"@"(yeast, control.group)
"@"(yeast, indices.optional)
"@"(yeast, fastq.gz.type)
```

RNASeqRParam-constructor

RNASeqRParam

Description

Constructor function for RNASeqRParam objects

Usage

```
RNASeqRParam(path.prefix = NA, input.path.prefix = NA,
  genome.name = NA, sample.pattern = NA, independent.variable = NA,
  case.group = NA, control.group = NA, fastq.gz.type = NA)
```

Arguments

path.prefix   Path prefix of 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/' and 'Rscript_out/' directories.

input.path.prefix

                    Path prefix of 'input_files/' directory.

genome.name   variable of genome name defined in this RNA-Seq workflow (ex. genome.name.fa, genome.name.gtf).

sample.pattern   Regular expression of paired-end fastq.gz files under 'input_files/raw_fastq.gz'. Expression not includes _[1,2].fastq.gz.

independent.variable

                    Independent variable for the biological experiment design of two-group RNA-Seq workflow.

case.group   Group name of the case group.

control.group   Group name of the control group.

fastq.gz.type   Specify the fastq.gz file type. 'PE' represents paired-end and 'SE' represents single-end.

Value

an object of class RNASeqRParam

Author(s)

kuan-hao Chao

Kuan-Hao Chao

Examples

```
input_files.path <- system.file("extdata/", package = "RNASeqRData")
rnaseq_result.path <- tempdir(check = TRUE)
exp <- RNASeqRParam(path.prefix         = rnaseq_result.path,
                    input.path.prefix   = input_files.path,
                    genome.name         = "Saccharomyces_cerevisiae_XV_Ensembl",
                    sample.pattern      = "SRR[0-9]*_XV",
                    independent.variable = "state",
                    case.group          = "60mins_ID20_amphotericin_B",
                    control.group       = "60mins_ID20_control",
                    fastq.gz.type       = "PE")
```

RNASeqRParam_Bam-class

RNASeqRParam_Bam

Description

An S4 class for checking and storing RNA-Seq workflow parameters starting with BAM files.

Slots

os.type 'linux' or 'osx'. The operating system type.

python.variable A list storing python environment. (check.answer,python.version)

python.2to3 Logical value whether 2to3 command is available on the workstation.

path.prefix Path prefix of 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/' and 'Rscript_out/' directories.

input.path.prefix Path prefix of 'input_files/' directory,

genome.name Variable of genome name defined in this RNA-Seq workflow (ex. genome.name.fa, genome.name.gtf).

sample.pattern Regular expression of paired-end fastq.gz files under 'input_files/raw_bam'. Expression not includes _[1,2].fastq.gz.

independent.variable Independent variable for the biological. experiment design of two-group RNA-Seq workflow.

case.group Group name of the case group.

control.group Group name of the control group.

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
"@"(yeast, os.type)
"@"(yeast, python.variable)
"@"(yeast, python.2to3)
"@"(yeast, path.prefix)
"@"(yeast, input.path.prefix)
"@"(yeast, genome.name)
"@"(yeast, sample.pattern)
"@"(yeast, independent.variable)
"@"(yeast, case.group)
"@"(yeast, control.group)
```

```
RNASeqRParam_Bam-constructor
```
                          RNASeqR_Bam

Description

Constructor function for RNASeqRParam_Bam objects

Usage

```
RNASeqRParam_Bam(path.prefix = NA, input.path.prefix = NA,
  genome.name = NA, sample.pattern = NA, independent.variable = NA,
  case.group = NA, control.group = NA)
```

Arguments

path.prefix   Path prefix of 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/' and
                 'Rscript_out/' directories.

input.path.prefix
                 Path prefix of 'input_files/' directory.

genome.name   variable of genome name defined in this RNA-Seq workflow (ex. genome.name.fa,
                 genome.name.gtf).

sample.pattern   Regular expression of paired-end fastq.gz files under 'input_files/raw_bam'. Ex-
                 pression not includes _[1,2].fastq.gz.

independent.variable
                 Independent variable for the biological experiment design of two-group RNA-
                 Seq workflow.

case.group   Group name of the case group.

control.group   Group name of the control group.

Value

an object of class `RNASeqRParam_Bam`

Author(s)

Kuan-Hao Chao

Kuan-Hao Chao

Examples

```
input_files.path <- system.file("extdata/", package = "RNASeqRData")
rnaseq_result.path <- tempdir(check = TRUE)
## Not run:
exp <- RNASeqRParam_Bam(path.prefix         = rnaseq_result.path,
                        input.path.prefix   = input_files.path,
                        genome.name         = "Saccharomyces_cerevisiae_XV_Ensembl",
                        sample.pattern      = "SRR[0-9]*_XV",
                        independent.variable = "state",
                        case.group          = "60mins_ID20_amphotericin_B",
                        control.group       = "60mins_ID20_control")

## End(Not run)
```

RNASeqRParam_Sam-class

RNASeqRParam_Sam

Description

An S4 class for checking and storing RNA-Seq workflow parameters starting with SAM files.

Slots

`os.type` 'linux' or 'osx'. The operating system type.

`python.variable` A list storing python environment. (`check.answer,python.version`)

`python.2to3` Logical value whether `2to3` command is available on the workstation.

`path.prefix` Path prefix of 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/' and 'Rscript_out/' directories.

`input.path.prefix` Path prefix of 'input_files/' directory,

`genome.name` Variable of genome name defined in this RNA-Seq workflow (ex. genome.name.fa, genome.name.gtf).

`sample.pattern` Regular expression of paired-end fastq.gz files under 'input_files/raw_sam'. Expression not includes `_[1,2].fastq.gz`.

`independent.variable` Independent variable for the biological. experiment design of two-group RNA-Seq workflow.

`case.group` Group name of the case group.

`control.group` Group name of the control group.

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
"@"(yeast, os.type)
"@"(yeast, python.variable)
"@"(yeast, python.2to3)
"@"(yeast, path.prefix)
"@"(yeast, input.path.prefix)
"@"(yeast, genome.name)
"@"(yeast, sample.pattern)
"@"(yeast, independent.variable)
"@"(yeast, case.group)
"@"(yeast, control.group)
```

RNASeqRParam_Sam-constructor

RNASeqR_Sam

Description

Constructor function for RNASeqRParam_Sam objects

Usage

```
RNASeqRParam_Sam(path.prefix = NA, input.path.prefix = NA,
  genome.name = NA, sample.pattern = NA, independent.variable = NA,
  case.group = NA, control.group = NA)
```

Arguments

path.prefix	Path prefix of 'gene_data/', 'RNASeq_bin/', 'RNASeq_results/', 'Rscript/' and 'Rscript_out/' directories.
input.path.prefix	
	Path prefix of 'input_files/' directory.
genome.name	variable of genome name defined in this RNA-Seq workflow (ex. genome.name.fa, genome.name.gtf).
sample.pattern	Regular expression of paired-end fastq.gz files under 'input_files/raw_sam'. Expression not includes _[1,2].fastq.gz.
independent.variable	
	Independent variable for the biological experiment design of two-group RNA-Seq workflow.
case.group	Group name of the case group.
control.group	Group name of the control group.

Value

an object of class RNASeqRParam_Sam

Author(s)

Kuan-Hao Chao

Kuan-Hao Chao

Examples

```
input_files.path <- system.file("extdata/", package = "RNASeqRData")
rnaseq_result.path <- tempdir(check = TRUE)
## Not run:
exp <- RNASeqRParam_Sam(path.prefix          = rnaseq_result.path,
                        input.path.prefix    = input_files.path,
                        genome.name          = "Saccharomyces_cerevisiae_XV_Ensembl",
                        sample.pattern       = "SRR[0-9]*_XV",
                        independent.variable = "state",
                        case.group           = "60mins_ID20_amphotericin_B",
                        control.group        = "60mins_ID20_control")

## End(Not run)
```

Update_Fastq_gz	*Update_Fastq_gz*

Description

This function let users update their trimmed fastq.gz files automatically.

Usage

```
Update_Fastq_gz(RNASeqRParam, prepared_fastq_gz, target_samples = "ALL")
```

Arguments

RNASeqRParam	S4 object instance of experiment-related parameters
prepared_fastq_gz	
	absolute path to the prepared 'raw_fastq.gz' directory.
target_samples	list of samples that are going to update. Default value is ALL

Value

None

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
## Not run:
RNASeqDifferentialAnalysis(RNASeqRParam = yeast)
## End(Not run)
```

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yeast   *Toy RNASeqRParam object*

Description

Small RNASeqRParam S4 object created with checked valid parameters for demonstration purposes

Author(s)

Kuan-Hao Chao

Examples

```
data(yeast)
yeast
# RNASeqRParam S4 object for example demonstration.
```

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