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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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, 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

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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/catal
`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/catal
`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 DGEList, 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 DGEList, 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 is`TRUE`. Set `FALSE` to skip 'HISAT2' installation.
`install.STAR`	Whether to install 'STAR' in this function step. Default value is`TRUE`. Set `FALSE` to skip 'STAR' installation.
`install.stringtie`	Whether to install 'StringTie' in this function step. Default value is `TRUE`. Set`FALSE` to skip 'StringTie' installation.
`install.gffcompare`	Whether to install 'Gffcompare' in this function step. Default value is `TRUE`. Set`FALSE` 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 is`TRUE`. Set `FALSE` to skip 'HISAT2' installation.
`install.STAR`	Whether to install 'STAR' in this function step. Default value is`TRUE`. Set `FALSE` to skip 'STAR' installation.
`install.stringtie`	Whether to install 'StringTie' in this function step. Default value is `TRUE`. Set`FALSE` to skip 'StringTie' installation.

`install.gffcompare`	Whether to install 'Gffcompare' in this function step. Default value is `TRUE`. Set`FALSE` 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/catalo
`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/catalo
`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 ballgown 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)

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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 ballgown 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'. 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_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)
```

`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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