

# Package ‘RNASeqR’

April 12, 2022

**Type** Package

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

**Version** 1.12.0

**Date** 2018-8-7

**Author** Kuan-Hao Chao

**Maintainer** Kuan-Hao Chao <ntueeb05howard@gmail.com>

**biocViews** Genetics, Infrastructure, DataImport, Sequencing, RNASeq, GeneExpression, GeneSetEnrichment, Alignment, QualityControl, DifferentialExpression, FunctionalPrediction, 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>

**git\_branch** RELEASE\_3\_14

**git\_last\_commit** 91e973c

**git\_last\_commit\_date** 2021-10-26

**Date/Publication** 2022-04-12

## R topics documented:

All_Steps_Interface . . . . .	3
All_Steps_Interface_CMD . . . . .	4
CheckToolAll . . . . .	6
RNASeqDifferentialAnalysis . . . . .	7
RNASeqDifferentialAnalysis_CMD . . . . .	8
RNASeqEnvironmentSet . . . . .	10
RNASeqEnvironmentSet_CMD . . . . .	12
RNASeqGoKegg . . . . .	13
RNASeqGoKegg_CMD . . . . .	15
RNASeqQualityAssessment . . . . .	16
RNASeqQualityAssessment_CMD . . . . .	17
RNASeqR . . . . .	18
RNASeqReadProcess . . . . .	18
RNASeqReadProcess_CMD . . . . .	27
RNASeqRParam-class . . . . .	37
RNASeqRParam-constructor . . . . .	38
RNASeqRParam_Bam-class . . . . .	39
RNASeqRParam_Bam-constructor . . . . .	40
RNASeqRParam_Sam-class . . . . .	41
RNASeqRParam_Sam-constructor . . . . .	42
Update_Fastq_gz . . . . .	43
yeast . . . . .	44
<b>Index</b>	<b>45</b>

---

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 package.

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/>

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 function 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 <a href="https://www.genome.jp/kegg/catalog/or">https://www.genome.jp/kegg/catalog/or</a>
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

<code>RNASeqRParam</code>	S4 object instance of experiment-related parameters
<code>which.trigger</code>	Default value is OUTSIDE. User should not change this value.
<code>INSIDE.path.prefix</code>	Default value is NA. User should not change this value.
<code>Pre_DE.visualization</code>	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

<code>RNASeqRParam</code>	S4 object instance of experiment-related parameters
<code>which.trigger</code>	Default value is OUTSIDE. User should not change this value.
<code>INSIDE.path.prefix</code>	Default value is NA. User should not change this value.
<code>Pre_DE.visualization</code>	Default TRUE. Whether to visualize pre-DE analysis results.
<code>Post_DE.visualization</code>	Default TRUE. Whether to visualize post-DE analysis results.
<code>ballgown.run</code>	Default TRUE. Logical value whether to run ballgown differential analysis.
<code>ballgown.pval</code>	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 <a href="https://www.genome.jp/kegg/catalog/or">https://www.genome.jp/kegg/catalog/or</a>
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 package.
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 <a href="https://www.genome.jp/kegg/catalog/or">https://www.genome.jp/kegg/catalog/or</a>
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.Assembly.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)**

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)
```

## 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.outQScnversionAdd  
 STAR alignment terminal '-outQScnversionAdd' 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**

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

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

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

# Index

All\_Steps\_Interface, [3](#)  
All\_Steps\_Interface\_CMD, [4](#)

CheckToolAll, [6](#)

RNASeqDifferentialAnalysis, [7](#)  
RNASeqDifferentialAnalysis\_CMD, [8](#)  
RNASeqEnvironmentSet, [10](#)  
RNASeqEnvironmentSet\_CMD, [12](#)  
RNASeqGoKegg, [13](#)  
RNASeqGoKegg\_CMD, [15](#)  
RNASeqQualityAssessment, [16](#)  
RNASeqQualityAssessment\_CMD, [17](#)  
RNASeqR, [18](#)  
RNASeqReadProcess, [18](#)  
RNASeqReadProcess\_CMD, [27](#)  
RNASeqRParam  
    (RNASeqRParam-constructor), [38](#)  
RNASeqRParam-class, [37](#)  
RNASeqRParam-constructor, [38](#)  
RNASeqRParam\_Bam  
    (RNASeqRParam\_Bam-constructor),  
    [40](#)  
RNASeqRParam\_Bam-class, [39](#)  
RNASeqRParam\_Bam-constructor, [40](#)  
RNASeqRParam\_Sam  
    (RNASeqRParam\_Sam-constructor),  
    [42](#)  
RNASeqRParam\_Sam-class, [41](#)  
RNASeqRParam\_Sam-constructor, [42](#)

Update\_Fastq\_gz, [43](#)

yeast, [44](#)