

# Package ‘CovidMutations’

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**Type** Package

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**Description** A feasible framework for mutation analysis and reverse transcription polymerase chain reaction (RT-PCR) assay evaluation of COVID-19, including mutation profile visualization, statistics and mutation ratio of each assay. The mutation ratio is conducive to evaluating the coverage of RT-PCR assays in large-sized samples. Mercatelli, D. and Giorgi, F. M. (2020) <[doi:10.20944/preprints202004.0529.v1](https://doi.org/10.20944/preprints202004.0529.v1)>.

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**BugReports** <https://github.com/MSQ-123/CovidMutations/issues>

**Suggests** testthat

**Author** Shaoqian Ma [aut, cre] (ORCID: <<https://orcid.org/0000-0001-8950-0711>>),  
Yongyou Zhang [aut] (ORCID: <<https://orcid.org/0000-0003-2413-9106>>)

**Maintainer** Shaoqian Ma <shaoqianma@qq.com>

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 AssayMutRatio

*Calculate the mutation detection rate using different assays*


---

### Description

This function is to use the well established assays information to detect mutations in different SARS-CoV-2 genomic sites. The output will be series of figures presenting the mutation profile using a specific assay and a figure for comparison between the mutation detection rate in each primers binding region.

### Usage

```
AssayMutRatio(
  nucmerr = nucmerr,
  assays = assays,
  totalsample = totalsample,
  plotType = "barplot",
  outdir = NULL
)
```

**Arguments**

nucmerr	Mutation information containing group list(derived from "nucmer" object using "nucmerRMD" function).
assays	Assays dataframe including the detection ranges of mutations.
totalsample	Total sample number, total cleared GISAID fasta data.
plotType	Figure type for either "barplot" or "logtrans".
outdir	The output directory.

**Value**

Plot the selected figure type as output.

**Examples**

```
data("nucmerr")
data("assays")
Total <- 52 ## Total Cleared GISAID fasta data, sekitseq
#outdir <- tempdir()
#Output the results
AssayMutRatio(nucmerr = nucmerr,
              assays = assays,
              totalsample = Total,
              plotType = "logtrans",
              outdir = NULL)
```

---

assays

*Assays for mutation detection using different primers and probes*

---

**Description**

These assays include the primer detection ranges in which mutations may occur.

**Usage**

```
data(assays)
```

**Format**

A dataframe with 10 rows and 7 columns.

**References**

Kilic T, Weissleder R, Lee H (2019) iScience 23, 101406. ([PubMed](#))

**Examples**

```
data(assays)
```

---

chinalist	<i>A list of places in China</i>
-----------	----------------------------------

---

**Description**

The list is used for displacing some original cities' names with "China" in order to make the downstream analysis easier.

**Usage**

```
data(chinalist)
```

**Format**

A dataframe with 31 rows and 1 column.

**Source**

This data is created by Zhanglab in Xiamen University.

**Examples**

```
data(chinalist)
```

---

covid_annot	<i>Mutation annotation results produced by "indelSNP" function</i>
-------------	--

---

**Description**

A dataframe which could be used for downstream analysis like mutation statistics description.

**Usage**

```
data(covid_annot)
```

**Format**

A dataframe with 394 rows and 10 columns.

**Source**

<https://www.gisaid.org/>

**Examples**

```
data(covid_annot)
```

---

`doubleAssay`*Detection of co-occurring mutations using double-assay information*

---

## Description

The detection of SARS-CoV-2 is important for the prevention of the outbreak and management of patients. Real-time reverse-transcription polymerase chain reaction (RT-PCR) assay is one of the most effective molecular diagnosis strategies to detect virus in clinical laboratory. It will be more accurate and practical to use double assays to detect some samples with co-occurring mutations.

## Usage

```
doubleAssay(nucmerr = nucmerr, assay1 = assay1, assay2 = assay2, outdir = NULL)
```

## Arguments

<code>nucmerr</code>	Mutation information containing group list(derived from "nucmer" object using "nucmerRMD" function).
<code>assay1</code>	Information of the first assay(containing primers locations and probe location, see the format of assays provided as example data. e.g. <code>data(assays)</code> ; <code>assay1&lt;-assays[1,]</code> )
<code>assay2</code>	Information of the second assay, the format is the same as the first assay.
<code>outdir</code>	The output directory. If NULL print the plot in Rstudio.

## Value

Plot three figures in a single panel, including two results of assays and a "venn" plot for co-occurring mutated samples.

## Examples

```
data("nucmerr")
data("assays")
assay1 <- assays[1,]
assay2 <- assays[2,]
#outdir <- tempdir()
doubleAssay(nucmerr = nucmerr,
            assay1 = assay1,
            assay2 = assay2,
            outdir = NULL)
```

---

gene_position	<i>"GFF3" format gene position data for SARS-Cov-2</i>
---------------	--

---

**Description**

This "GFF3" data is used for counting the mutations in each gene in virus sample.

**Usage**

```
data(gene_position)
```

**Format**

A dataframe with 26 rows and 10 columns.

**Source**

<https://www.ncbi.nlm.nih.gov/>

**Examples**

```
data(gene_position)
```

---

gff3	<i>"GFF3" format annotation data for SARS-Cov-2</i>
------	---

---

**Description**

This "GFF3" data is used for annotating the effects of mutations in virus sample.

**Usage**

```
data(gff3)
```

**Format**

A dataframe with 26 rows and 10 columns.

**Source**

<https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=2697049>

**Examples**

```
data(gff3)
```

---

globalProteinMut	<i>Global mutational events profiling of proteins</i>
------------------	---

---

## Description

This function is to visualize the global protein mutational pattern in the SARS-CoV-2 genome.

## Usage

```
globalProteinMut(  
  covid_annot = covid_annot,  
  outdir = NULL,  
  figure_Type = "heatmap",  
  top = 10,  
  country = "global"  
)
```

## Arguments

covid_annot	The mutation effects provided by "indelSNP" function.
outdir	The output directory.
figure_Type	Figure type for either "heatmap" or "count".
top	The number of variants to plot.
country	Choose a country to plot the mutational pattern or choose "global" to profile mutations across all countries. The default is "global".

## Value

Plot the selected figure type as output.

## Examples

```
data("covid_annot")  
outdir <- tempdir()  
# make sure the covid_annot is a dataframe  
covid_annot <- as.data.frame(covid_annot)  
globalProteinMut(covid_annot = covid_annot,  
  outdir = outdir,  
  figure_Type = "heatmap",  
  top = 10,  
  country = "USA")
```

---

globalSNPprofile	<i>Global single nucleotide polymorphism (SNP) profiling in virus genome</i>
------------------	--

---

### Description

This function is to visualize the global SNP pattern in the SARS-CoV-2 genome.

### Usage

```
globalSNPprofile(  
  nucmerr = nucmerr,  
  outdir = NULL,  
  figure_Type = "heatmap",  
  country = "global",  
  top = 5  
)
```

### Arguments

nucmerr	Mutation information containing group list(derived from "nucmer" object using "nucmerRMD" function).
outdir	The output directory.
figure_Type	Figure type for either "heatmap" or "count".
country	Choose a country to plot the mutational pattern or choose "global" to profile mutations across all countries. The default is "global".
top	The number of mutational classes to plot.

### Value

Plot the selected figure type as output.

### Examples

```
data("nucmerr")  
outdir <- tempdir()  
globalSNPprofile(nucmerr = nucmerr,  
  outdir = outdir,  
  figure_Type = "heatmap",  
  country = "global",  
  top = 5)
```

---

indelSNP	<i>Provide effects of each single nucleotide polymorphism (SNP), insertion and deletion in virus genome</i>
----------	---

---

## Description

This function is to annotate the mutational events and indicate their potential effects on the proteins. Mutational events include SNP, insertion and deletion.

## Usage

```
indelSNP(
  nucmer = nucmer,
  saveRda = FALSE,
  refseq = refseq,
  gff3 = gff3,
  annot = annot,
  outdir = NULL
)
```

## Arguments

nucmer	An object called "nucmer", mutation information derived from "nucmer.snp" variant file by "seqkit" software and "nucmer SNP-calling" scripts. To be processed by "indelSNP" function, The nucmer object should be first transformed by "mergeEvents" function.
saveRda	Whether to save the results as ".rda" file.
refseq	SARS-Cov-2 genomic reference sequence.
gff3	"GFF3" format annotation data for SARS-Cov-2.
annot	Annotation of genes(corresponding proteins) list from "GFF3" file by "setNames(gff3[,10],gff3[,9])".
outdir	The output directory.

## Value

Write the result as ".csv" file to the specified directory.

## Examples

```
data("nucmer")
# Fix IUPAC codes
nucmer<-nucmer[!nucmer$qvar%in%c("B","D","H","K","M","N","R","S","V","W","Y"),]
nucmer<- mergeEvents(nucmer = nucmer)## This will update the nucmer object
data("refseq")
data("gff3")
annot <- setNames(gff3[,10],gff3[,9])
#outdir <- tempdir()
```

```
nucmer<- indelSNP(nucmer = nucmer,  
                 saveRda = FALSE,  
                 refseq = refseq,  
                 gff3 = gff3,  
                 annot = annot,  
                 outdir = NULL)
```

---

LastfiveNrMutation      *Bacth assay analysis for last five Nr of primers*

---

## Description

Last five nucleotides of primer mutation count/type for any reverse transcription polymerase chain reaction (RT-PCR) primer.

## Usage

```
LastfiveNrMutation(  
  nucmerr = nucmerr,  
  assays = assays,  
  totalsample = totalsample,  
  figurelist = FALSE,  
  outdir = NULL  
)
```

## Arguments

nucmerr	Mutation information containing group list(derived from "nucmer" object using "nucmerRMD" function).
assays	Assays dataframe including the detection ranges of mutations.
totalsample	Total sample number, total cleared GISAID fasta data.
figurelist	Whether to output the integrated plot list for each assay.
outdir	The output directory. if the figurelist = TRUE, output the figure in the R session.

## Value

Plot the mutation counts(last five nucleotides for each primer) for each assay as output.

## Examples

```
data("nucmerr")  
data("assays")  
totalsample <- 434  
#outdir <- tempdir()  
LastfiveNrMutation(nucmerr = nucmerr,  
                  assays = assays,  
                  totalsample = totalsample,  
                  figurelist = FALSE,  
                  outdir = NULL)
```

---

mergeEvents	<i>Merge neighboring events of single nucleotide polymorphism (SNP), insertion and deletion.</i>
-------------	--

---

### Description

The first step for handling the nucmer object, then effects of mutations can be analysed using "indelSNP" function.

### Usage

```
mergeEvents(nucmer = nucmer)
```

### Arguments

nucmer	An object called "nucmer", mutation information derived from "nucmer.snp" variant file by "seqkit" software and "nucmer SNP-calling" scripts.
--------	---

### Value

An updated "nucmer" object.

### Examples

```
#The example data:
data("nucmer")
#options(stringsAsFactors = FALSE)

#The input nucmer object can be made by the comment below:
#nucmer<-read.delim("nucmer.snps",as.is=TRUE,skip=4,header=FALSE)
#colnames(nucmer)<-c("rpos","rvar","qvar","qpos","","","","","","rlength","qlength","","","rname","qname")
#rownames(nucmer)<-paste0("var",1:nrow(nucmer))

# Fix IUPAC codes
nucmer<-nucmer[!nucmer$qvar%in%c("B","D","H","K","M","N","R","S","V","W","Y"),]
nucmer<- mergeEvents(nucmer = nucmer)## This will update the nucmer object
```

---

MutByGene	<i>Plot mutation counts for certain genes</i>
-----------	---

---

### Description

After annotating the mutations, this function is to plot the counts of mutational events for each gene in the SARS-CoV-2 genome.

**Usage**

```
MutByGene(nucmerr = nucmerr, gff3 = gff3, figurelist = FALSE, outdir = NULL)
```

**Arguments**

nucmerr	Mutation information containing group list(derived from "nucmer" object using "nucmerRMD" function).
gff3	"GFF3" format gene position data for SARS-Cov-2(the "GFF3" file should include columns named: "Gene", "Start", "Stop").
figurelist	Whether to output the integrated plot list for each gene.
outdir	The output directory, if the figurelist = TRUE, output the figure in the R session.

**Value**

Plot the mutation counts figure for each gene as output.

**Examples**

```
data("nucmerr")
data("gene_position")
#outdir <- tempdir()
MutByGene(nucmerr = nucmerr, gff3 = gene_position, figurelist = FALSE, outdir = NULL)
#if figurelist = TRUE, the recommendation for figure display(in pixel)is: width=1650, height=1300
```

---

mutStat

*Plot mutation statistics for nucleotide*


---

**Description**

Visualization for the top mutated samples, average mutational counts, top mutated position in the genome, mutational density across the genome and distribution of mutations across countries.

**Usage**

```
mutStat(
  nucmerr = nucmerr,
  outdir = NULL,
  figure_Type = "TopMuSample",
  type_top = 10,
  country = FALSE,
  mutpos = NULL
)
```

**Arguments**

nucmerr	Mutation information containing group list(derived from "nucmer" object using "nucmerRMD" function).
outdir	The output directory.
figure_Type	Figure type for: "TopMuSample", "AverageMu", "TopMuPos", "MutDens", "CountryMutCount", "TopCountryMut".
type_top	To plot the figure involving "top n"("TopMuSample", "TopMuPos", "TopCountryMut"), the "type_top" should specify the number of objects to display.
country	To plot the figure using country as groups("CountryMutCount" and "TopCountryMut"), the "country" should be TRUE.
mutpos	If the figure type is "TopCountryMut", "mutpos" can specify A range of genomic position(eg. 28831:28931) for plot

**Value**

Plot the selected figure type as output.

**Examples**

```
data("nucmerr")
outdir <- tempdir()
mutStat(nucmerr = nucmerr,
        outdir = outdir,
        figure_Type = "TopCountryMut",
        type_top = 10,
        country = FALSE,
        mutpos = NULL)
```

---

nucmer

---

*Mutation information derived from "nucmer" SNP analysis*


---

**Description**

The "nucmer.snps" variant file is obtained by processing the SARS-Cov-2 sequence from Gisaidd website (complete, high coverage only, low coverage exclusion, Host=human, Virus name = hCoV-19) with "seqkit" software and "nucmer" scripts. The example data is downsampled from complete data in 2020-07-28 (0.001 proportion, 52 samples).

**Usage**

```
data(nucmer)
```

**Format**

A dataframe with 437 rows (mutation sites) and 14 columns.

**Source**

<https://www.gisaid.org/>

**Examples**

```
data(nucmer)
```

---

nucmerr

*Preprocessed "nucmer.snps" file using "nucmerRMD" function*

---

**Description**

A dataset contains some group information subtracted from the "nucmer" object by "nucmerRMD" function in order to best describe the results.

**Usage**

```
data(nucmerr)
```

**Format**

A dataframe with 437 rows (downsampled mutation sites) and 10 columns.

**Source**

<https://www.gisaid.org/>

**Examples**

```
data(nucmerr)
```

---

nucmerRMD

*Preprocess "nucmer" object to add group information*

---

**Description**

Manipulate the "nucmer" object to make the analysis easier.

**Usage**

```
nucmerRMD(nucmer = nucmer, outdir = NULL, chinalist = chinalist)
```

**Arguments**

nucmer	An object called "nucmer", mutation information derived from "nucmer.snp" variant file by "seqkit" software and "nucmer SNP-calling" scripts.
outdir	The output directory.
chinalist	A list of places in China, for displacing some original cities with "China" in order to make the downstream analysis easier.

**Value**

Saving the updated "nucmer" object.

**Examples**

```
data("nucmer")
data("chinalist")
#outdir <- tempdir()
nucmerRMD(nucmer = nucmer, outdir = NULL, chinalist = chinalist)
```

---

plotMutAnno	<i>Plot the mutation statistics after annotating the "nucmer" object by "indelSNP" function</i>
-------------	---

---

**Description**

Basic descriptions for the mutational events.

**Usage**

```
plotMutAnno(covid_annot = covid_annot, figureType = "MostMut", outdir = NULL)
```

**Arguments**

covid_annot	The mutation effects provided by "indelSNP" function.
figureType	Figure type for: "MostMut", "MutPerSample", "VarClasses", "VarType", "NucleoEvents", "ProEvents".
outdir	The output directory.

**Value**

Plot the selected figure type as output.

**Examples**

```
data("covid_annot")
# make sure the covid_annot is a dataframe
covid_annot <- as.data.frame(covid_annot)
#outdir <- tempdir() specify your output directory
plotMutAnno(covid_annot = covid_annot, figureType = "MostMut", outdir = NULL)
```

---

plotMutProteins	<i>Plot the most frequent mutational events for proteins in the SARS-CoV-2 genome</i>
-----------------	---

---

### Description

Plot the most frequent mutational events for proteins selected. The protein name should be specified correctly (only for SARS-CoV-2).

### Usage

```
plotMutProteins(  
  covid_annot = covid_annot,  
  proteinName = "NSP2",  
  top = 20,  
  outdir = NULL  
)
```

### Arguments

covid_annot	The mutation effects provided by "indelSNP" function.
proteinName	Proteins in the SARS-CoV-2 genome, available choices: 5'UTR, NSP1~NSP10, NSP12a, NSP12b, NSP13, NSP14, NSP15, NSP16, S, ORF3a, E, M, ORF6, ORF7a, ORF7b, ORF8, N, ORF10.
top	The number of objects to display.
outdir	The output directory.

### Value

Plot the mutational events for selected proteins as output.

### Examples

```
data("covid_annot")  
# make sure the covid_annot is a dataframe  
covid_annot <- as.data.frame(covid_annot)  
#outdir <- tempdir() specify your output directory  
plotMutProteins(covid_annot = covid_annot,proteinName = "NSP2", top = 20, outdir = NULL)
```

---

`refseq`*SARS-Cov-2 genomic reference sequence from NCBI*

---

**Description**

This reference sequence is derived from "fasta" file, preprocessed by "read.fasta" function(`refseq<-read.fasta("NC_045512.2.fa",forceDNAtolower=FALSE)[[1]]`). It is used for annotating mutations in virus samples.

**Usage**

```
data(refseq)
```

**Format**

"SeqFastadna" characters.

**Source**

<https://pubmed.ncbi.nlm.nih.gov/32015508/>

**Examples**

```
data(refseq)
```

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