

# Package ‘scMAGeCK’

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**Type** Package

**Title** Identify genes associated with multiple expression phenotypes in single-cell CRISPR screening data

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**Description**

scMAGeCK is a computational model to identify genes associated with multiple expression phenotypes from CRISPR screening coupled with single-cell RNA sequencing data (CROP-seq)

**License** BSD\_2\_clause

**biocViews** CRISPR, SingleCell, RNASeq, PooledScreens, Transcriptomics, GeneExpression, Regression

**NeedsCompilation** yes

**Imports** Seurat, ggplot2, stats, utils

**Suggests** knitr, rmarkdown

**VignetteBuilder** knitr

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scMAGeCK-package	<i>Identify genes associated with multiple expression phenotypes in single-cell CRISPR screening data</i>
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**Description**

scMAGeCK is a computational model to identify genes associated with multiple expression phenotypes from CRISPR screening coupled with single-cell RNA sequencing data (CROP-seq)

**Details**

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scMAGeCK is a computational model to identify genes associated with multiple expression phenotypes from CRISPR screening coupled with single-cell RNA sequencing data (CROP-seq). scMAGeCK is based on our previous MAGeCK and MAGeCK-VISPR models for pooled CRISPR screens.

The scMAGeCK manuscript can be found at bioRxiv(<https://www.biorxiv.org/content/10.1101/658146v1/>).

**Author(s)**

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**Examples**

```
### BARCODE file contains cell identity information, generated from
### the cell identity collection step
BARCODE <- system.file("extdata", "barcode_rec.txt", package = "scMAGeCK")
### RDS can be a Seurat object or local RDS file path that contains
### the scRNA-seq dataset
RDS <- system.file("extdata", "singles_dox_mki67_v3.RDS", package = "scMAGeCK")
```

```
target_gene <- "MKI67"
```

```
### Set RRA executable file path or activate scmageck env if needed (see https://bitbucket.org/weililab/scmageck)
RRAPATH <- NULL
```

```
rra_result <- scmageck_rra(BARCODE=BARCODE, RDS=RDS, GENE=target_gene,
                           RRAPATH=RRAPATH, LABEL='dox_mki67',
                           NEGCTRL=NULL, KEPTMP=FALSE,
                           PATHWAY=FALSE, SAVEPATH=NULL)
```

```
head(rra_result)
```

```
lr_result <- scmageck_lr(BARCODE=BARCODE, RDS=RDS, LABEL='dox_scmageck_lr',
                        NEGCTRL = 'NonTargetingControlGuideForHuman', PERMUTATION = 1000,
                        SAVEPATH=NULL, LAMBDA=0.01)
```

```
lr_score <- lr_result[1]
lr_score_pval <- lr_result[2]
head(lr_score_pval)
```

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featurePlot	<i>Detect the sgRNA distribution and generate Vlnplot to identity gene regulation between different cells.</i>
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## Description

identify how many sgRNAs entered the cells, visualize the counts of sgRNAs distribution and gene regulation.

## Usage

```
featurePlot(RDS, TYPE = plot.type, BARCODE = NULL, sgRNA = NULL, GENE = NULL, CONTROL = NULL, palette = N

plot.type
# c("Dis", "Vln", "Den")
```

## Arguments

RDS	RDS object from the pre-processRDS step
TYPE	Type of the plot.
BARCODE	A txt file to include cell identity information, generated from the cell identity collection step.
sgRNA	Generate whole sgRNAs distribution when sgRNA = NULL, add sgRNAs to see the specific sgRNA distribution. Mutiple sgRNAs can be provided, separated by ",". For example, "APC,TP53".
GENE	Genes whose expressions are to be compared under different cell coditions. Multiple genes can be provided, separated by ",". For example, "APC,TP53". when provide mutiple genes, it would show the average gene expression.
CONTROL	Set up the sepecific clusters to compare the gene expression. it would compare gene expression across the dataset when CONTROL = NULL.
palette	The color palette to change the color of VlnPlot.
label.size	Text size of label.
axis.size	Text size of axis.text.
title.size	Text size of axis/pics' title.
legend.text	Text size of figure legend.
fill	Fill colour.

## Examples

```
### Loading required package
require(Seurat)

### BARCODE file contains cell identity information, generated from the cell identity collection step
BARCODE <- system.file("extdata", "barcode_rec.txt", package = "scMAGeCK")

### RDS can be a Seurat object or local RDS file path that contains the scRNA-seq dataset
RDS <- system.file("extdata", "singles_dox_mki67_v3.RDS", package = "scMAGeCK")

### For using the featurePlot function, it needs to do the preprocessRDS first
Demo <- pre_processRDS(BARCODE = BARCODE, RDS = RDS)

### For the sgRNA distribution
featurePlot(BARCODE = BARCODE, RDS = Demo, TYPE = "Dis")

### For the density of sgRNA, clustering needed to be done first.
Demo <- RunUMAP(Demo, dims = 1:10)
featurePlot(RDS = Demo, sgRNA = NULL, TYPE = "Den")

### For the Vlnplot to display the gene regulation, take MKI67 for examples
featurePlot(RDS = Demo, GENE = "MKI67", sgRNA = "TP53", TYPE = "Vln")
```

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pre_processRDS	<i>Integrate the information of sgRNA into RDS file for the further analysis.</i>
----------------	---

---

## Description

Pre-process the sgRNA count from previous step, and generate the sgRNA expression matrix.

## Usage

```
pre_processRDS(BARCODE, RDS)
```

## Arguments

BARCODE	A txt file to include cell identity information, generated from the cell identity collection step.
RDS	A Seurat object or local RDS file path that contains the scRNA-seq dataset. Note that the dataset has to be normalized and scaled.

## Examples

```
### BARCODE file contains cell identity information, generated from the cell identity collection step
BARCODE <- system.file("extdata", "barcode_rec.txt", package = "scMAGeCK")

### RDS can be a Seurat object or local RDS file path that contains the scRNA-seq dataset
RDS <- system.file("extdata", "singles_dox_mki67_v3.RDS", package = "scMAGeCK")
```

```
Demo <- pre_processRDS(BARCODE = BARCODE, RDS = RDS)
```

---

scmageck_lr	<i>Use linear regression to test the association of gene knockout with all possible genes</i>
-------------	---

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## Description

```
echo "Use linear regression to test the association of gene knockout with all possible genes"
```

## Usage

```
scmageck_lr(BARCODE, RDS, NEGCTRL, SELECT_GENE=NULL, LABEL = NULL,
PERMUTATION = NULL, SIGNATURE = NULL, SAVEPATH = "./", LAMBDA=0.01, GENE_FRAC=0.01)
```

## Arguments

BARCODE	A txt file to include cell identity information, generated from the cell identity collection step.
RDS	A Seurat object or local RDS file path that contains the scRNA-seq dataset. Note that the dataset has to be normalized and scaled.
NEGCTRL	The name of the genes (separated by ",") served as negative controls.
SELECT_GENE	The list of genes for regression. By default, all genes in the table are subject to regression.
LABEL	The label of the output file.
PERMUTATION	The number of permutations for p value calculation.
SAVEPATH	The save path of result. Default save path is the current working directory. If you don't need save the result, set SAVEPATH as NULL.
LAMBDA	A paramter for the LR model for ridge regression. Default: 0.01.
GENE_FRAC	A paramter for filtering low expressed genes. By default, only genes that have expressions in at least that fractions of cells (in raw count table) are kept. If raw count table is not found, will look into scaled expression, and only keep genes whose expression is greater than zero in at least that fraction of cells. Default: 0.01.
SIGNATURE	A GMT text file, the format must be as follows:(1)Column 1: name of gene set; (2)Column 2: Empty, or the information about gene set e.g. the source of the gene set; (3)Column 3 and onwards: ids of genes belonging to a particular gene set. Note that this parameter for applying LR model for the gene set analysis. Reference: <a href="http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats">http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats</a>

## Value

The result for object RDS

## Examples

```
### BARCODE file contains cell identity information, generated from the cell identity collection step
BARCODE <- system.file("extdata","barcode_rec.txt",package = "scMAGECK")

### RDS can be a Seurat object or local RDS file path that contains the scRNA-seq dataset
RDS <- system.file("extdata","singles_dox_mki67_v3.RDS",package = "scMAGECK")

lr_result <- scmageck_lr(BARCODE=BARCODE, RDS=RDS, LABEL='dox_scmageck_lr', SIGNATURE = NULL,
  NEGCTRL = 'NonTargetingControlGuideForHuman', PERMUTATION = 1000, SAVEPATH=NULL, LAMBDA=0.01)
lr_score <- lr_result[1]
lr_score_pval <- lr_result[2]
head(lr_score_pval)
```

---

scmageck_rra	<i>Use RRA to test the association of gene knockout with certain marker expression</i>
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---

## Description

echo "Use RRA to test the association of gene knockout with certain marker expression"

## Usage

```
scmageck_rra(BARCODE, RDS, GENE, RRAPATH = NULL, LABEL = NULL, NEGCTRL = NULL,
SIGNATURE = NULL, KEPTMP = FALSE, PATHWAY = FALSE, SAVEPATH = "./")
```

## Arguments

BARCODE	A txt file to include cell identity information, generated from the cell identity collection step.
RDS	A Seurat object or local RDS file path that contains the scRNA-seq dataset. Note that the dataset has to be normalized and scaled.
GENE	Genes whose expressions are to be tested. Multiple genes can be provided, separated by ",". For example, "MKI67,TP53"
RRAPATH	The path to the RRA program, if RRA cannot be found in the PATH environment variable.
LABEL	The label of the output file.
NEGCTRL	The name of the negative control gene. For example, "NonTargetingControl-GuideForHuman". Default is NULL (do not use any negative controls).
KEPTMP	Keep temporary files.
PATHWAY	Treat genes in –GENE option as a pathway. In other words, the averaged expression of these genes will be used for testing.
SAVEPATH	The save path of result. Default save path is the current working directory. If you don't need save the result, set SAVEPATH as NULL.

**SIGNATURE** A GMT text file, the format must be as follows:(1)Column 1: name of gene set; (2)Column 2: Empty, or the information about gene set e.g. the source of the gene set; (3)Column 3 and onwards: ids of genes belonging to a particular gene set. Note that if you don't set the parameter "SAVEPATH", this parameter would create a folder called "GENE\_SET"" in the current working directory to store the results from applying RRA program to do gene set analysis. Reference:[http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data\\_formats](http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats)

## Value

The result for object RDS

## Examples

```
### BARCODE file contains cell identity information, generated from the cell identity collection step
BARCODE <- system.file("extdata", "barcode_rec.txt", package = "scMAGECK")

### RDS can be a Seurat object or local RDS file path that contains the scRNA-seq dataset
RDS <- system.file("extdata", "singles_dox_mki67_v3.RDS", package = "scMAGECK")

target_gene <- "MKI67"
### Set RRA executable file path or activate scmageck env if needed (see https://bitbucket.org/weililab/scmageck)
RRAPATH <- NULL

rra_result <- scmageck_rra(BARCODE=BARCODE, RDS=RDS, GENE=target_gene,
                           RRAPATH=RRAPATH, LABEL='dox_mki67',
                           NEGCTRL=NULL, KEEPTMP=FALSE, SIGNATURE = NULL,
                           PATHWAY=FALSE, SAVEPATH=NULL)

head(rra_result)
```

---

selectPlot

*generate the selection plot*


---

## Description

detect the gene regulation relationship between genes and perturbation by using RRA or LR test.

## Usage

```
selectPlot(GENE = NULL, lr_result = NULL, CUTOFF = 0.05, ADJ = "fdr",
           RRA_re1 = NULL, RRA_re2 = NULL, TYPE = select.type, QUALITY = 10)

select.type
#c("lr", "rra")
```

**Arguments**

GENE	Genes whose expressions are to be tested under the LR test. Multiple genes can be provided, separated by ",". For example, "MKI67,TP53"
lr_result	The result from the scmageck-lr step.
CUTOFF	Determine the significant pvalue.
ADJ	P.adjust.methods. Choose one of correction method. c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none")
RRA_re1	RRA result from the scmageck-rra step.
RRA_re2	Optional input. The second RRA result from the scmageck-rra step. Add this input to visualize gene selection under two different cell condition.
TYPE	The type of the scMAGeCK results. Can be either "rra" or "lr".
QUALITY	The number of single-cells that passes the threshold when use the RRA test, default is 10. Lower quality could improve the sensitivity but reduce accuracy.

**Examples**

```

### by using RRA test, take MKI67 for example
### only works if you have RRA installed
#selectPlot(RRA_re1 = rra_result, CUTOFF = 0.05, QUALITY = 10, ADJ = "fdr", TYPE = "rra")

### by using LR test, take MKI67 for example

### BARCODE file contains cell identity information, generated from the cell identity collection step
BARCODE <- system.file("extdata", "barcode_rec.txt", package = "scMAGeCK")

### RDS can be a Seurat object or local RDS file path that contains the scRNA-seq dataset
RDS <- system.file("extdata", "singles_dox_mki67_v3.RDS", package = "scMAGeCK")

lr_result <- scmageck_lr(BARCODE=BARCODE, RDS=RDS, LABEL='dox_scmageck_lr', SIGNATURE = NULL,
  NEGCTRL = 'NonTargetingControlGuideForHuman', PERMUTATION = 1000, SAVEPATH=NULL, LAMBDA=0.01)
selectPlot(GENE = "MKI67", lr_result = lr_result, CUTOFF = 0.05, ADJ = "fdr", TYPE = "lr")

```



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