# Package 'cypress'

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Type Package

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**Suggests** knitr, rmarkdown, MatrixGenerics, htmltools, RUnit, BiocGenerics, BiocManager, BiocStyle, Biobase

Collate AllClasses.R AllGenerics.R CIBERSORT.R csRNA\_seq\_sim.R cypress\_design.R cypress\_wrapper.R cypressEmbedded.R metrics.R cypress-methods.R PlotTDR.R DE\_arg.R TOAST\_implement.R user.R util\_datasim.R util\_EST.R PlotFDC.R PlotPower.R

biocViews Software, GeneExpression, DataImport, RNASeq, Sequencing

Description CYPRESS is a cell-type-specific power tool. This package aims to perform power analysis for the cell-type-specific data. It calculates FDR, FDC, and power, under various study design parameters, including but not limited to sample size, and effect size. It takes the input of a SummarizeExperimental(SE) object with observed mixture data (feature by sample matrix), and the cell-type mixture proportions (sample by cell-type matrix). It can solve the cell-type mixture proportions from the reference free panel from TOAST and conduct tests to identify cell-type-specific differential expression (csDE) genes.

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## Description

The **cypress** package is specifically designed to perform comprehensive cell-type-specific power assessment for differential expression using RNA-sequencing experiments. It accepts real Bulk RNAseq data as input for parameter estimation and simulation. The tool provides flexibility by allowing users to customize sample sizes, number of cell types, and effect sizes (log-fold change). Additionally, it computes statistical power, true discovery rate (TDR), and false discovery cost (FDC) under different scenarios as results.

## **Details**

**cypress** is the first statistical tool to evaluate the power in cell-type-specific Differentially Expressed (csDE) genes detection experiments from a prospective way by letting researchers be flexible in tuning sample sizes, effect sizes, csDE genes percentage, total number of genetic features, type I error control, etc.

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#### Value

cypress offers 3 options for simulation and power evaluation: simFromData(), simFromParam() and quickPower(). If users have their own bulk RNA-seq count data, they can use the simFromData() function; otherwise, they can use simFromParam(), which uses one of the three sets of simulation parameters estimated from existing studies, to perform power evaluation under user-defined simulation settings. If users prefer to quickly examine the power evaluation results and do not want to run the simulation, they can use the quickPower() function to view results from our existing simulations. The output of these 3 functions is a S4 object including a list of simulation results under various experimental settings, including as statistical power, TDR, and FDC.

Once users have obtained an S4 object with a list of results output from either simFromData(), simFromParam() or quickPower(), they can use the following functions to generate basic evaluation plots: plotPower(), plotTDR() and plotFDC().

#### Author(s)

Shilin Yu <sy597@georgetown.edu> Guanqun Meng <gxm324@case.edu> Wen Tang <wxt175@case.edu>

asd\_nopropPower

Power calculation results From ASD data

## **Description**

Pre-calculated power evaluation results from Autism Spectrum Disorder (ASD) study. The results can be used to create plots using plot functions (plotFDC, plotPower, plotTDR).

#### Usage

```
data('quickPowerASD')
```

## Format

```
A S4 object.
```

ct\_TDR\_bio\_smry Cell-type-specific target TDR.

TDR\_bio\_smry Average target TDR.

ct\_PWR\_bio\_smry Cell-type-specific target power.

PWR\_bio\_smry Average target power.

PWR\_strata\_ct\_bio\_smry Cell type specific target power by gene expression stratification.

PWR\_strata\_bio\_smry Average target power by gene expression stratification.

ct\_FDC\_bio\_smry Cell type specific target FDC.

FDC\_bio\_smry Average target FDC.

#### Value

One S4 object.

```
data('quickPowerASD')
```

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asd\_noprop\_param

Simulation parameters estimated from ASD study

## **Description**

An S4 object that stores parameter estimates associated with the Autism Spectrum Disorder (ASD) dataset. This object contains a variety of numerical vectors and matrices representing different statistical parameters used in the simulation.

# Usage

```
data('quickParaASD')
```

#### **Format**

Simulation parameters for simFromParam function.

- health\_lm\_mean A numeric vector containing the log-mean parameter estimates for each cell type from healthy samples.
- health\_lm\_mean\_d A matrix containing the variance-covariance estimates of log-mean parameters across cell types from healthy samples.
- lod\_m A numeric vector containing the log-dispersion parameter estimates for each cell type from healthy samples.
- lod\_d A matrix containing the variance-covariance estimates of log-dispersion parameters across cell types from healthy samples.
- health\_alpha A numeric vector of the estimated alpha parameter used to simulate cell type proportions for healthy samples.
- case\_alpha A numeric vector of the estimated alpha parameter used to simulate cell type proportions for case samples.

# Value

One S4 object.

```
data('quickParaASD')
```

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• • •	ASD_prop	SimFromData example raw input data	
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## **Description**

ASD\_prop is an example of SummarizedExperiment (SE) object input for the simFromData function. It contains the following elements:

counts A gene expression value dataset from Autism Spectrum Disorder (ASD) study, in the form of raw read counts, 29674 genes by 48 samples, with 24 cases and 24 controls

colData Sample meta-data. The first column is the group status (i.e. case/ctrl), the second column is the subject ID. The remaining are the cell type proportions of all samples.

## Usage

```
data(ASD_prop_se)
```

#### **Format**

SE object.

#### Value

One SE object.

## **Examples**

data(ASD\_prop\_se)

cypress-class

Classes for cypress Package

# Description

The cypress\_out and est\_out classes are custom S4 classes in the **cypress** package. both classes are designed as a comprehensive container for various types of analysis results.

## Value

Class for cypress.

## cypress\_out

**Description:** The cypress\_out class is a S4 class in the **cypress** package. This class is customized to better present results and use for cypress plot functions.

#### **Slots:**

```
ct_TDR_bio_smry Cell type specific target TDR
TDR_bio_smry Target TDR
ct_PWR_bio_smry Cell type specific target power
```

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```
PWR_bio_smry Target power

PWR_strata_bio_smry Target power by gene expression stratification

PWR_strata_ct_bio_smry Cell type specific target power by gene expression stratification

ct_FDC_bio_smry Cell type specific target FDC

FDC_bio_smry Target FDC.
```

# est\_out

**Description:** The est\_out class is designed to output the parameter estimated results, providing a structured representation of results.

## **Slots:**

```
health_alpha Control group proportion simulation parameter.

case_alpha Case group proportion simulation parameter.

health_lmean_m Mean of genetic distribution mean for each cell.

health_lmean_d Var/cov of genetic distribution mean among cell types

lod_m Mean of genetic distribution dispersion for each cell.

lod_d Var/cov of genetic distribution dispersion among cell types.

sample_CT_prop Matrix of sample Cell type proportions.

genename Gene Name.

samplename Sample Name.

CTname Cell type names.

dimensions_Z_hat_ary dimensions for the Z hat array.
```

## Author(s)

Shilin Yu <sy597@georgetown.edu>

#### **Examples**

```
data(quickParaGSE60424)
```

cypress-methods

Get a slot from cypress output

## **Description**

Accessor function for getting or replace slots. Show methods for cypress object.

## Usage

```
getcypress(object, name)
setcypress(object, name, value)
```

# **Arguments**

object from cypress.

name name of the slot in cypress object.
value value of the slot in cypress object.

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#### Value

Methods for cypress.

## **Examples**

```
data(quickPowerIBD)
getcypress(ibd_propPower, "ct_TDR_bio_smry")
```

GSE60424Power

Power calculation results from immune-related disease (IAD) study (GSE60424)

## Description

Pre-calculated power evaluation results from immune-related disease (IAD) study (GSE60424). The results can be used to create plots using plot functions (plotFDC, plotPower, plotTDR).

## Usage

```
data('quickPowerGSE60424')
```

## **Format**

```
A S4 object.
```

ct\_TDR\_bio\_smry Cell-type-specific target TDR.

TDR\_bio\_smry Average target TDR.

 $\verb|ct_PWR_bio_smry| Cell-type-specific target power.$ 

PWR\_bio\_smry Average target power.

PWR\_strata\_ct\_bio\_smry Cell type specific target power by gene expression stratification.

PWR\_strata\_bio\_smry Average target power by gene expression stratification.

ct\_FDC\_bio\_smry Cell type specific target FDC.

FDC\_bio\_smry Average target FDC.

#### Value

One S4 object.

```
data('quickPowerGSE60424')
```

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GSE60424_param	Simulation parameters estimated from immune-related disease (IAD) study (GSE60424)

## **Description**

An S4 object that stores simulation parameter estimated from the immune-related disease (IAD) study (GSE60424). This object contains a variety of numerical vectors and matrices representing different statistical parameters used in the simulation. The patients were drawn from healthy subjects from the immune-related diseases study.

## Usage

```
data('quickParaGSE60424')
```

#### **Format**

Simulation parameters for simFromParam function.

- health\_lm\_mean A numeric vector containing the log-mean parameter estimates for each cell type from healthy samples.
- health\_lm\_mean\_d A matrix containing the variance-covariance estimates of log-mean parameters across cell types from healthy samples.
- lod\_m A numeric vector containing the log-dispersion parameter estimates for each cell type from healthy samples.
- lod\_d A matrix containing the variance-covariance estimates of log-dispersion parameters across cell types from healthy samples.
- health\_alpha A numeric vector of the estimated alpha parameter used to simulate cell type proportions for healthy samples.
- case\_alpha A numeric vector of the estimated alpha parameter used to simulate cell type proportions for case samples.

#### Value

One S4 object.

```
data('quickParaGSE60424')
```

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ibd_propPower	Power calculation results from pediatric inflammatory bowel disease (IBD) study (GSE57945)
---------------	--

## **Description**

Pre-calculated power evaluation results from pediatric inflammatory bowel disease (IBD) study (GSE57945). The results can be used to create plots using plot functions (plotFDC, plotPower, plotTDR).

## Usage

```
data('quickPowerIBD')
```

#### **Format**

```
A S4 object.
```

ct\_TDR\_bio\_smry Cell-type-specific target TDR.

TDR\_bio\_smry Average target TDR.

ct\_PWR\_bio\_smry Cell-type-specific target power.

PWR\_bio\_smry Average target power.

PWR\_strata\_ct\_bio\_smry Cell type specific target power by gene expression stratification.

PWR\_strata\_bio\_smry Average target power by gene expression stratification.

ct\_FDC\_bio\_smry Cell type specific target FDC.

FDC\_bio\_smry Average target FDC.

#### Value

One S4 object.

## **Examples**

```
data('quickPowerIBD')
```

ibd\_prop\_param

Simulation parameters estimated from pediatric inflammatory bowel disease (IBD) study (GSE57945)

## **Description**

An S4 object that stores simulation parameter estimated from the inflammatory bowel disease (IBD) study (GSE57945). This object contains a variety of numerical vectors and matrices representing different statistical parameters used in the simulation. The patients were drawn from healthy subjects from the immune-related diseases study.

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#### Usage

```
data('quickParaIBD')
```

#### **Format**

Simulation parameters for simFromParam function.

health\_lm\_mean A numeric vector containing the log-mean parameter estimates for each cell type from healthy samples.

health\_lm\_mean\_d A matrix containing the variance-covariance estimates of log-mean parameters across cell types from healthy samples.

lod\_m A numeric vector containing the log-dispersion parameter estimates for each cell type from healthy samples.

lod\_d A matrix containing the variance-covariance estimates of log-dispersion parameters across cell types from healthy samples.

health\_alpha A numeric vector of the estimated alpha parameter used to simulate cell type proportions for healthy samples.

case\_alpha A numeric vector of the estimated alpha parameter used to simulate cell type proportions for case samples.

#### Value

One S4 object.

## **Examples**

data('quickParaIBD')

plotFDC

plotFDC: Generate FDC results figure

## Description

Plot false discovery cost results. This function plots false discovery cost results in a 2x1 panel. The illustration of each plot from left to right:

- 1: False discovery cost(FDC) by effect size, each line represents cell type. Sample size to be fixed at 10 if sample\_size=10.
- 2: False discovery cost(FDC) by top effect size, each line represents sample size. FDC was the average value across cell types.

## **Arguments**

simulation\_results

A list of results produced by power evaluation functions.

sample\_size

A numerical value indicating which sample size to be fixed. For example, 10 means when plotting the relationship between FDC and effect size, we fixed the scenario of sample size at 10. Default is 10.

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#### Value

This function does not return a value. It generates a two-panel plot visualizing the false discovery cost (FDC) results. The first panel shows the FDC by effect size for each cell type at a fixed sample size (default is 10). The second panel illustrates the FDC by the top effect sizes, with each line representing a different sample size, averaged across cell types.

### Author(s)

Wen Tang <wxt175@case.edu> Shilin Yu <sy597@georgetown.edu>

#### **Examples**

```
data(quickPowerGSE60424)
### Plot power results
plotFDC(GSE60424Power,sample_size=10)
```

plotPower

plotPower: Generate statistical power results figure

## **Description**

This function plots all statistical power measurements in a 2x3 panel. The illustration of each plot from left to right and from up to bottom is as follows:

- 1: Statistical power by effect size, each line represents sample size. Statistical power was the average value across cell types.
- 2: Statistical power by effect size, each line represents cell type. Sample size is fixed at 10 if sample\_size=10.
- 3: Statistical power by sample size, each line represents cell type. Effect size is fixed at 1 if effect.size=1.
- 4: Statistical power by strata, each line represents cell type. Sample size is fixed at 10 and effect size is fixed at 1 if sample\_size=10 and effect.size=1.
- 5: Statistical power by strata, each line represents sample size. Statistical power was the average value across cell types and effect size is fixed at 1 if effect.size=1.
- 6: Statistical power by strata, each line represents effect size. Statistical power was the average value across cell types and sample size is fixed at 10 if sample\_size=10.

#### **Arguments**

simulation\_results

A list of results produced by power evaluation functions.

effect.size A numerical value indicating which effect size is to be fixed. For example, 1 means when plotting the relationship between power and strata, we fixed the

scenario of log fold change at 1. The default is 1.

sample\_size A numerical value indicating which sample size to be fixed. For example, 10 means when plotting the relationship between power and strata, we fixed the

scenario of sample size at 10. The default is 10.

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#### Value

This function generates a 2x3 panel plot visualizing various statistical power measurements, but does not return a programmable value. Each panel displays power metrics under different conditions such as effect size, sample size, and stratification, with lines representing either sample size, cell type, or effect size.

#### Author(s)

Wen Tang <wxt175@case.edu> Shilin Yu <sy597@georgetown.edu>

## **Examples**

```
data(quickPowerGSE60424)
### Plot power results
plotPower(GSE60424Power,effect.size=1,sample_size=10)
```

plotTDR

plotTDR: Generate TDR results figure

#### **Description**

This function plots all true discovery rate measurements in a 2x2 panel. The illustration of each plot is as follows:

- 1: True discovery rate(TDR) by top-rank genes, each line represents cell type. Sample size to be fixed at 10 and effect size to be fixed at 1 if sample\_size=10 and effect.size=1.
- 2: True discovery rate(TDR) by top rank genes, each line represents effect size. TDR was the average value across cell types and sample size is fixed at 10 if sample\_size=10.
- 3: True discovery rate(TDR) by top rank genes, each line represents sample size. TDR was the average value across cell types and effect size is fixed at 1 if effect.size=1.
- 4: True discovery rate(TDR) by effect size, each line represents sample size. TDR was calculated under the scenario of top rank gene equals 350.

## Arguments

simulation\_results

A list of results produced by power evaluation functions.

effect.size

A numerical value indicating which effect size is to be fixed. For example, 1 means when plotting the relationship between TDR and top rank genes for cell types or sample size, we fixed the scenario of log2 fold change at 1. The default is 1

sample\_size

A numerical value indicating which sample size to be fixed. For example, 10 means when plotting the relationship between TDR and top rank genes for cell types or effect size, we fixed the scenario of sample size at 10. The default is 10.

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#### Value

This function creates a 2x2 panel plot showcasing various true discovery rate (TDR) measurements but does not return any values for further programmatic use. Each panel displays TDR analyses based on top rank genes, with lines representing different cell types, effect sizes, or sample sizes under specific conditions.

#### Author(s)

Wen Tang <wxt175@case.edu> Shilin Yu <sy597@georgetown.edu>

#### **Examples**

```
data(quickPowerGSE60424)
### Plot power results
plotTDR(GSE60424Power,effect.size=1,sample_size=10)
```

quickPower

Obtain pre-calculated results from three available datasets

## **Description**

This function quickly outputs pre-calculated power evaluation results from three datasets: (IAD, IBD, and ASD). The obtained results can be used to create plots from plot functions.

#### Usage

```
quickPower(data = "IAD")
```

## **Arguments**

data

A character string specifying the dataset to be retrieved. Options are 'IAD', 'IBD', and 'ASD'.

#### **Details**

- IAD: Whole transcriptome signatures of 6 immune cell subsets. The patients were drawn from subjects with a range of immune-related diseases.
- IBD: Inflammatory Bowel Disease
- ASD: Autism Spectrum Disorder.

### Value

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## Author(s)

Shilin Yu <sy597@georgetown.edu> Guanqun Meng <gxm324@case.edu>

## **Examples**

```
# library(cypress)
Quick_power <- quickPower(data = "IAD")</pre>
```

simFromData

Power calculation results on input data

## **Description**

This function conducts simulations with various user-defined study design parameters, Users will need to provide SE object bulk data for parameter estimation purposes.

# Usage

## Arguments

INPUTdata	The input SE (SummarizedExperiment) object should contain a count matrix, study design, and an optional cell type proportion matrix. The study design should have a column named 'disease', where the control by 1 and the case is indicated by 2. If provided, the cell type proportion matrix should sum to 1 for each sample. The cell type proportion matrix is optional, the CT_unk should be True if the user did not provide this matrix
CT_index	Column index for cell types proportion matrix in Coldata, the input can also be a single number (>3) when the CT_unk is T.
CT_unk	Logical flag indicating whether unknown cell types are present. Defaults to FALSE.
n_sim	The total number of iterations users wish to conduct. Default to 3. In simulation results, it is set to 20.
n_gene	Total number of genetic features users with to conduct. Default to 30000. Must be greater than or equal to 1000.
DE_pct	Percentage of DEG on each cell type. Default to 0.05.

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ss_group_set	Sample sizes per group users wish to simulate. The length should be less than or equal to 5. Default to 10,20,50.
lfc_set	Effect sizes users wish to simulate. The length should be less than or equal to 5. Default to 0,0.5,1,1.5.
lfc_target	Target effect size, should be greater than or equal to 0. The absolute LFC lower than this values will be treated as None-DEGs. Default to 0.5
fdr_thred	Adjusted p value threshold. The parameter value should be within the range (0, 1). Default to 0.1
DEmethod	Differential expression (DE) methods available include 'TOAST', 'DESeq2', and 'CeDAR'. The default method is 'TOAST'
BPPARAM	An instance of BiocParallelParam class, e.g. MulticoreParam, SnowParam, SerialParam, to facilitate parallel computing. If using Unix, MulticoreParam is recommended. Customized options within BiocParallelParam class is allowed. If not specified, the default back-end is retrieve.

#### **Details**

One SummarizedExperiment object containing the following elements:

```
counts A gene expression value dataset
```

colData Sample meta-data. The first column is the group status (i.e. case/ctrl) named as 'disease', and the second column is the subject ID. The remaining are the cell type proportions of all samples. The user could also input the Column index for cell types proportion matrix in Coldata. Example: CT\_index= 3:8

#### Value

```
ct_TDR_bio_smry
                  Cell-type-specific target TDR.
TDR_bio_smry
                  Average target TDR.
ct_PWR_bio_smry
                  Cell-type-specific target power.
PWR_bio_smry
                  Average target power.
PWR_strata_ct_bio_smry
                  Cell type specific target power by gene expression stratification.
PWR_strata_bio_smry
                  Average target power by gene expression stratification.
ct_FDC_bio_smry
                  Cell type specific target FDC.
FDC_bio_smry
                  Average target FDC.
```

#### Author(s)

Shilin Yu <sy597@georgetown.edu> Guanqun Meng <gxm324@case.edu>

```
data(ASD_prop_se)
result <- simFromData(INPUTdata = ASD_prop, CT_index = (seq_len(6) + 2),
CT_unk = FALSE, n_sim = 2,n_gene = 1000, DE_pct = 0.05,
ss_group_set = c(8,10), lfc_set = c(1, 1.5))</pre>
```

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simFromParam	Power calculation results on pre-calculated parameters	

# Description

This function conducts simulations with various user-defined study design parameters, including but not limited to sample size, and log fold change.)

# Usage

## **Arguments**

guinents	
n_sim	The total number of iterations users wish to conduct. Default to 3. In simulation results, it is set to 20.
n_gene	Total number of genetic features users with to conduct. Default to 30000. Must be greater than or equal to 1000.
DE_pct	Percentage of DEG on each cell type. Default to 0.05.
ss_group_set	Sample sizes per group users wish to simulate. The length should be less than or equal to 5. Default to 10,20,50.
lfc_set	effect sizes users wish to simulate. The length should be less than or equal to 5. Default to 0,0.5,1,1.5.
sim_param	Users specify which embedded simulation parameters they wish to use. By default set to 'IAD', which is a cell line specific data. Other options include 'IBD' data, and 'ASD' data
lfc_target	Target effect size, should be greater than or equal to $0$ . The absolute LFC lower than this value will be treated as None-DEGs. Default to $0.5$
fdr_thred	Adjusted p value threshold. The parameter value should be within the range (0, 1). Default to 0.1
DEmethod	Differential expression (DE) methods available include 'TOAST', 'DESeq2', and 'CeDAR'. The default method is 'TOAST'
BPPARAM	An instance of BiocParallelParam class, e.g. MulticoreParam, SnowParam, SerialParam, to facilitate parallel computing. If using Unix, MulticoreParam is recommended. Customized options within BiocParallelParam class is allowed. If not specified, the default back-end is retrieve.

## **Details**

- GSE60424: Immune-related disease (IAD) study. Whole transcriptome signatures of 6 immune cell subsets. The patients were drawn from subjects with a range of immune-related diseases.
- IBD: data in pediatric inflammatory bowel disease(IBD) study
- ASD: data in a large autism spectrum disorder (ASD) study

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## Value

```
ct_TDR_bio_smry
                  Cell-type-specific target TDR.
                  Average target TDR.
TDR_bio_smry
ct_PWR_bio_smry
                  Cell-type-specific target power.
PWR_bio_smry
                  Average target power.
PWR_strata_ct_bio_smry
                  Cell type specific target power by gene expression stratification.
PWR_strata_bio_smry
                  Average target power by gene expression stratification.
ct_FDC_bio_smry
                  Cell type specific target FDC.
                  Average target FDC.
FDC_bio_smry
```

## Author(s)

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