

Package ‘CytoGLMM’

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Type Package

Title Conditional Differential Analysis for Flow and Mass Cytometry Experiments

Version 1.14.0

Description The CytoGLMM R package implements two multiple regression strategies: A bootstrapped generalized linear model (GLM) and a generalized linear mixed model (GLMM). Most current data analysis tools compare expressions across many computationally discovered cell types. CytoGLMM focuses on just one cell type. Our narrower field of application allows us to define a more specific statistical model with easier to control statistical guarantees. As a result, CytoGLMM finds differential proteins in flow and mass cytometry data while reducing biases arising from marker correlations and safeguarding against false discoveries induced by patient heterogeneity.

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URL <https://christofseiler.github.io/CytoGLMM>,
<https://github.com/ChristofSeiler/CytoGLMM>

BugReports <https://github.com/ChristofSeiler/CytoGLMM/issues>

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LazyData true

Imports stats, methods, BiocParallel, RColorBrewer, cowplot,
doParallel, dplyr, factoextra, flexmix, ggplot2, magrittr,
mbest, pheatmap, stringr, strucchange, tibble, ggrepel, MASS,
logging, Matrix, tidyr, caret, rlang, grDevices

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Software

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cytoflexmix	<i>Logistic mixture regression</i>
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Description

Logistic mixture regression

Usage

```
cytoflexmix(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  cell_n_min = Inf,
  cell_n_subsample = 0,
  ks = seq_len(10),
  num_cores = 1
)
```

Arguments

df_samples_subset	Data frame or tibble with proteins counts, cell condition, and group information
protein_names	A vector of column names of protein to use in the analysis
condition	The column name of the condition variable
group	The column name of the group variable
cell_n_min	Remove samples that are below this cell counts threshold
cell_n_subsample	Subsample samples to have this maximum cell count
ks	A vector of cluster sizes
num_cores	Number of computing cores

Value

A list of class `cytoglm` containing

flexmixfits	list of <code>flexmix</code> objects
df_samples_subset	possibly subsampled <code>df_samples_subset</code> table
protein_names	input protein names
condition	input condition variable
group	input group names
cell_n_min	input <code>cell_n_min</code>
cell_n_subsample	input <code>cell_n_subsample</code>
ks	input <code>ks</code>
num_cores	input <code>num_cores</code>

Examples

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
mix_fit <- CytoGLMM::cytoflexmix(df,
                                protein_names = protein_names,
                                condition = "condition",
                                group = "donor",
                                ks = 2)

mix_fit
```

cytoglm

*Fit GLM with bootstrap resampling***Description**

Fit GLM with bootstrap resampling

Usage

```
cytoglm(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  covariate_names = NULL,
  cell_n_min = Inf,
  cell_n_subsample = 0,
  num_boot = 100,
  num_cores = 1
)
```

Arguments

df_samples_subset	Data frame or tibble with proteins counts, cell condition, and group information
protein_names	A vector of column names of protein to use in the analysis
condition	The column name of the condition variable
group	The column name of the group variable
covariate_names	The column names of covariates
cell_n_min	Remove samples that are below this cell counts threshold
cell_n_subsample	Subsample samples to have this maximum cell count
num_boot	Number of bootstrap samples
num_cores	Number of computing cores

Value

A list of class cytoglm containing

tb_coef	coefficient table
df_samples_subset	possibly subsampled df_samples_subset table
protein_names	input protein names
condition	input condition variable
group	input group names
covariate_names	input covariates

```

cell_n_min      input cell_n_min
cell_n_subsample
                 input cell_n_subsample

unpaired        true if unpaired samples were provided as input
num_boot        input num_boot
num_cores       input num_cores
formula_str     formula use in the regression model

```

Examples

```

set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
glm_fit <- CytoGLMM::cytoglm(df,
                             protein_names = protein_names,
                             condition = "condition",
                             group = "donor",
                             num_boot = 10) # in practice >=1000

glm_fit

```

cytoglmm

Fit GLMM with method of moments

Description

Fit GLMM with method of moments

Usage

```

cytoglmm(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  covariate_names = NULL,
  cell_n_min = Inf,
  cell_n_subsample = 0,
  num_cores = 1
)

```

Arguments

```

df_samples_subset      Data frame or tibble with proteins counts, cell condition, and group information
protein_names          A vector of column names of protein to use in the analysis
condition              The column name of the condition variable
group                  The column name of the group variable
covariate_names        The column names of covariates

```

cell_n_min Remove samples that are below this cell counts threshold
 cell_n_subsample Subsample samples to have this maximum cell count
 num_cores Number of computing cores

Value

A list of class `cytoglm` containing

glmmfit `mbest` object
 df_samples_subset possibly subsampled `df_samples_subset` table
 protein_names input protein names
 condition input condition variable
 group input group names
 covariate_names input covariates
 cell_n_min input `cell_n_min`
 cell_n_subsample input `cell_n_subsample`
 num_cores input `num_cores`

Examples

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
glmm_fit <- CytoGLMM::cytoglmm(df,
                               protein_names = protein_names,
                               condition = "condition",
                               group = "donor")

glmm_fit
```

cytogroup

Group-specific fixed effects model

Description

Group-specific fixed effects model

Usage

```
cytogroup(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  cell_n_min = Inf,
  cell_n_subsample = 0
)
```

Arguments

df_samples_subset	Data frame or tibble with proteins counts, cell condition, and group information
protein_names	A vector of column names of protein to use in the analysis
condition	The column name of the condition variable
group	The column name of the group variable
cell_n_min	Remove samples that are below this cell counts threshold
cell_n_subsample	Subsample samples to have this maximum cell count

Value

A list of class `cytoglm` containing

groupfit	<code>glm</code> object
df_samples_subset	possibly subsampled <code>df_samples_subset</code> table
protein_names	input protein names
condition	input condition variable
group	input group names
cell_n_min	input <code>cell_n_min</code>
cell_n_subsample	input <code>cell_n_subsample</code>

Examples

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
group_fit <- CytoGLMM::cytogroup(df,
                                protein_names = protein_names,
                                condition = "condition",
                                group = "donor")

group_fit
```

 cytostab

Evaluate parameter stability with respect to gating scheme

Description

Evaluate parameter stability with respect to gating scheme

Usage

```

cytostab(
  df_samples_subset,
  protein_names,
  condition,
  group = "donor",
  cell_n_min = Inf,
  cell_n_subsample = 0
)

```

Arguments

```

df_samples_subset      Data frame or tibble with proteins counts, cell condition, and group information
protein_names          A vector of column names of protein to use in the analysis
condition              The column name of the condition variable
group                  The column name of the group variable
cell_n_min             Remove samples that are below this cell counts threshold
cell_n_subsample       Subsample samples to have this maximum cell count

```

Value

A data frame

Examples

```

set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
stab <- CytoGLMM::cytostab(df,
                           protein_names = protein_names,
                           condition = "condition",
                           group = "donor")

stab

```

cyto_check

Check if input to cytoxxx function have errors

Description

Check if input to cytoxxx function have errors

Usage

```
cyto_check(cell_n_subsample, cell_n_min, protein_names)
```


Arguments

cell_n_subsample Subsample samples to have this maximum cell count
cell_n_min A vector of column names of protein to use in the analysis
protein_names A vector of column names of protein to use in the analysis

Value

NULL.

generate_data	<i>Generate dataset for vignettes and simulation studies</i>
---------------	--

Description

Generate dataset for vignettes and simulation studies

Usage

```
generate_data()
```

Value

`tibble` data frame

Examples

```
set.seed(23)  
df <- generate_data()  
str(df)  
df
```

glmm_moment	<i>Generalized linear mixed model with maximum likelihood</i>
-------------	---

Description

Generalized linear mixed model with maximum likelihood

Usage

```
glmm_moment(  
  df_samples,  
  protein_names,  
  response,  
  group = "donor",  
  covariate_names = NULL,  
  num_cores = 1  
)
```

Arguments

df_samples	Data frame or tibble with proteins counts, cell condition, and group information
protein_names	A vector of column names of protein to use in the analysis
response	The column name of the condition variable
group	The column name of the group variable
covariate_names	The column names of covariates
num_cores	Number of computing cores

Value

`mbest` object

is_unpaired	<i>Check if samples match or paired on condition</i>
-------------	--

Description

Check if samples match or paired on condition

Usage

```
is_unpaired(df_samples_subset, condition, group)
```

Arguments

df_samples_subset	Data frame or tibble with proteins counts, cell condition, and group information
condition	The column name of the condition variable
group	The column name of the group variable

Value

A boolean

plot.cytoflexmix	<i>Plot all components of mixture regression</i>
------------------	--

Description

Plot all components of mixture regression

Usage

```
## S3 method for class 'cytoflexmix'
plot(x, k = NULL, separate = FALSE, ...)
```

Arguments

x	A cytoflexmix class
k	Number of clusters
separate	create two separate ggplot2 objects
...	Other parameters

Value

[ggplot2](#) object

Examples

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
mix_fit <- CytoGLMM::cytoflexmix(df,
                                protein_names = protein_names,
                                condition = "condition",
                                group = "donor",
                                ks = 2)

plot(mix_fit)
```

plot.cytoglm *Plot bootstraped coefficients*

Description

Plot bootstraped coefficients

Usage

```
## S3 method for class 'cytoglm'
plot(x, order = FALSE, separate = FALSE, ...)
```

Arguments

x	A cytoglm class
order	Order the markers according to the mangintute of the coefficients
separate	create two separate ggplot2 objects
...	Other parameters

Value

[ggplot2](#) object

plot.cytogroup	<i>Plot fixed coefficients of group-specific fixed effects model</i>
----------------	--

Description

Plot fixed coefficients of group-specific fixed effects model

Usage

```
## S3 method for class 'cytgroup'
plot(x, order = FALSE, separate = FALSE, ...)
```

Arguments

x	A cytoglmm class
order	Order the markers according to the magnitude of the coefficients
separate	create two separate <code>ggplot2</code> objects
...	Other parameters

Value

`ggplot2` object

Examples

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
group_fit <- CytoGLMM::cytgroup(df,
                                protein_names = protein_names,
                                condition = "condition",
                                group = "donor")

plot(group_fit)
```

plot_coeff	<i>Helper function to plot regression coefficient</i>
------------	---

Description

Helper function to plot regression coefficient

Usage

```
plot_coeff(
  tb,
  title_str,
  title_str_right,
  xlab_str,
  redline = 0,
  order = FALSE,
  separate = FALSE
)
```

Arguments

tb	A data frame
title_str	Title string for summary plot
title_str_right	Title for bootstrap sample plot
xlab_str	Label on x-axis
redline	Point on x-axis to draw the red line
order	Order the markers according to the magnitude of the coefficients
separate	Plot both summary and bootstrap samples

Value

[ggplot2](#) object or list of two objects if separate is true

plot_heatmap	<i>Heatmap of median marker expression</i>
--------------	--

Description

Heatmap of median marker expression

Usage

```
plot_heatmap(
  df_samples,
  sample_info_names,
  protein_names,
  arrange_by_1,
  arrange_by_2 = "",
  cluster_cols = FALSE,
  fun = median
)
```

Arguments

df_samples	Data frame or tibble with proteins counts, cell condition, and group information
sample_info_names	Column names that contain information about the cell, e.g. donor, condition, file name, or cell type
protein_names	A vector of column names of protein to use in the analysis
arrange_by_1	Column name
arrange_by_2	Column name
cluster_cols	Apply hierarchical cluster to columns
fun	Summary statistics of marker expression

Value

`pheatmap` object

Examples

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
CytoGLMM::plot_heatmap(df,
                        protein_names = protein_names,
                        sample_info_names = c("donor", "condition"),
                        arrange_by_1 = "condition")
```

plot_lda

LDA on marker expression

Description

LDA on marker expression

Usage

```
plot_lda(
  df_samples,
  protein_names,
  group,
  cor_scaling_factor = 1,
  arrow_color = "black",
  marker_color = "black",
  marker_size = 5
)
```

Arguments

`df_samples` Data frame or tibble with proteins counts, cell condition, and group information
`protein_names` A vector of column names of protein to use in the analysis
`group` The column name of the group variable
`cor_scaling_factor` Scaling factor of circle of correlations
`arrow_color` Color of correlation circle
`marker_color` Colors of marker names
`marker_size` Size of marker names

Value

`ggplot2` object

Examples

```

set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
df$condition <- rep(c("A", "B", "C", "D"), each = length(df$condition)/4)
CytoGLMM::plot_lda(df,
                    protein_names = protein_names,
                    group = "condition",
                    cor_scaling_factor = 2)
  
```

plot_mds

MDS on median marker expression

Description

MDS on median marker expression

Usage

```

plot_mds(
  df_samples,
  protein_names,
  sample_info_names,
  color,
  sample_label = ""
)
  
```

Arguments

`df_samples` Data frame or tibble with proteins counts, cell condition, and group information
`protein_names` A vector of column names of protein to use in the analysis
`sample_info_names` Column names that contain information about the cell, e.g. donor, condition, file name, or cell type
`color` Column name
`sample_label` Column name

Value

cowplot object

Examples

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
CytoGLMM::plot_mds(df,
                    protein_names = protein_names,
                    sample_info_names = c("donor", "condition"),
                    color = "condition")
```

plot_model_selection *Plot model selection to choose number optimal number of clusters*

Description

Plot model selection to choose number optimal number of clusters

Usage

```
plot_model_selection(fit, k = NULL)
```

Arguments

fit	A cytoflexmix class
k	Number of clusters

Value

cowplot object

Examples

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
mix_fit <- CytoGLMM::cytoflexmix(df,
                                protein_names = protein_names,
                                condition = "condition",
                                group = "donor",
                                ks = 1:2)

plot_model_selection(mix_fit)
```

plot_prcomp *Plot PCA of subsampled data using ggplot*

Description

Plot PCA of subsampled data using ggplot

Usage

```
plot_prcomp(
  df_samples,
  protein_names,
  color_var = "treatment",
  subsample_size = 10000,
  repel = TRUE
)
```

Arguments

`df_samples` Data frame or tibble with proteins counts, cell condition, and group information
`protein_names` A vector of column names of protein to use in the analysis
`color_var` A column name
`subsample_size` Subsample per `color_var` variable
`repel` Repel labels

Value

`cowplot` object

Examples

```
set.seed(23)
df <- generate_data()
protein_names <- names(df)[3:12]
df <- dplyr::mutate_at(df, protein_names, function(x) asinh(x/5))
CytoGLMM::plot_prcomp(df,
  protein_names = protein_names,
  color_var = "condition")
```

print.cytoglm *Extact and print bootstrap GLM fit*

Description

Extact and print bootstrap GLM fit

Usage

```
## S3 method for class 'cytoglm'
print(x, ...)
```

remove_samples	<i>Remove samples based on low cell counts</i>
----------------	--

Description

Remove samples based on low cell counts

Usage

```
remove_samples(df_samples_subset, condition, group, unpaired, cell_n_min)
```

Arguments

df_samples_subset	Data frame or tibble with proteins counts, cell condition, and group information
condition	The column name of the condition variable
group	The column name of the group variable
unpaired	true if unpaired samples were provided as input
cell_n_min	Remove samples that are below this cell counts threshold

Value

NULL.

summary.cytoglm	<i>Extact and calculate p-values of bootstrap GLM fit</i>
-----------------	---

Description

Extact and calculate p-values of bootstrap GLM fit

Usage

```
## S3 method for class 'cytoglm'
summary(object, method = "BH", ...)
```

Arguments

object	A cytoglm class
method	Multiple comparison adjustment method
...	Other parameters

Value

[tibble](#) data frame

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