

Package ‘CytoGLMM’

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Type Package

Title Conditional Differential Analysis for Flow and Mass Cytometry Experiments

Version 1.16.0

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

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Contents

cytoflexmix	2
cytoglm	4
cytogroup	5
cytostab	7
cyto_check	8
generate_data	8
is_unpaired	9
plot.cytoflexmix	9
plot.cytoglm	10
plot.cytogroup	11
plot_coeff	11
plot_heatmap	12
plot_lda	13
plot_mds	14
plot_model_selection	15
plot_prcomp	16
print.cytoglm	17
remove_samples	18
summary.cytoglm	18
Index	20

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

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

Value

A list of class `cytoglm` containing

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

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

Value

A list of class `cytoglm` containing

<code>tb_coef</code>	coefficient table
<code>df_samples_subset</code>	possibly subsampled <code>df_samples_subset</code> table
<code>protein_names</code>	input protein names
<code>condition</code>	input condition variable
<code>group</code>	input group names
<code>covariate_names</code>	input covariates
<code>cell_n_min</code>	input <code>cell_n_min</code>
<code>cell_n_subsample</code>	input <code>cell_n_subsample</code>
<code>unpaired</code>	true if unpaired samples were provided as input
<code>num_boot</code>	input <code>num_boot</code>
<code>num_cores</code>	input <code>num_cores</code>
<code>formula_str</code>	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
```

cytgroup	<i>Group-specific fixed effects model</i>
----------	---

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

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

Value

A list of class `cytoglm` containing

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

<code>df_samples_subset</code>	Data frame or tibble with proteins counts, cell condition, and group information
<code>protein_names</code>	A vector of column names of protein to use in the analysis
<code>condition</code>	The column name of the condition variable
<code>group</code>	The column name of the group variable
<code>cell_n_min</code>	Remove samples that are below this cell counts threshold
<code>cell_n_subsample</code>	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	<i>Check if input to cytoxxx function have errors</i>
------------	---

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
```

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 bootstrapped coefficients***Description**

Plot bootstrapped 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

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

plot(glm_fit)

```

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 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))
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 mangintute of the coefficients
separate	Plot both summary and bootstrap samples

Value

ggplot2 object or list of two objects if separate is true

plot_heatmap	Heatmap of median marker expression
--------------	-------------------------------------

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

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

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 markerr 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	<i>Extact and print bootstrap GLM fit</i>
---------------	---

Description

Extact and print bootstrap GLM fit

Usage

```

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

```

Arguments

x	A cytoglm class
...	Other parameters

Value

NULL.

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

print(glm_fit)

```

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>Extract and calculate p-values of bootstrap GLM fit</i>
-----------------	--

Description

Extract 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

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
summary(glm_fit)
```

Index

cowplot, [15](#), [16](#)
cyto_check, [8](#)
cytoflexmix, [2](#)
cytoglm, [4](#)
cytogroup, [5](#)
cytostab, [7](#)

flexmix, [3](#)

generate_data, [8](#)
ggplot2, [9–12](#), [14](#)
glm, [6](#)

is_unpaired, [9](#)

pheatmap, [13](#)
plot.cytoflexmix, [9](#)
plot.cytoglm, [10](#)
plot.cytogroup, [11](#)
plot_coeff, [11](#)
plot_heatmap, [12](#)
plot_lda, [13](#)
plot_mds, [14](#)
plot_model_selection, [15](#)
plot_prcomp, [16](#)
print.cytoglm, [17](#)

remove_samples, [18](#)

summary.cytoglm, [18](#)

tibble, [8](#), [18](#)