Package: CytoGLMM (via r-universe)

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Type Package

Title Conditional Differential Analysis for Flow and Mass Cytometry Experiments

Version 1.22.0

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

Encoding UTF-8

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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`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.

---

cytoflexmix *Logistic mixture regression*

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 `flexmix` objects
`df_samples_subset`	possibly subsampled df_samples_subset table
`protein_names`	input protein names
`condition`	input condition variable
`group`	input group names
`cell_n_min`	input cell_n_min
`cell_n_subsample`	input cell_n_subsample
`ks`	input ks
`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))
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	coefficent 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`	glm object
`df_samples_subset`	possibly subsampled df_samples_subset table
`protein_names`	input protein names
`condition`	input condition variable
`group`	input group names
`cell_n_min`	input cell_n_min
`cell_n_subsample`	input cell_n_subsample

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 sheme*

Description

Evaluate parameter stability with respect to gating sheme

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
```

---

generate_data *Generate dataset for vignettes and simulation studies*

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 *Generalized linear mixed model with maximum likelihood*

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 *Check if samples match or paired on condition*

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 *Plot all components of mixture regression*

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

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.cytoglmm *Plot fixded coefficients of random effects model*

Description

Plot fixded coefficients of random effects model

Usage

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

Arguments

x	A cytoglmm 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))
glmm_fit <- CytoGLMM::cytoglmm(df,
                               protein_names = protein_names,
                               condition = "condition",
                               group = "donor")
plot(glmm_fit)
```

---

plot.cytogroup *Plot fixded coefficients of group-specific fixed effects model*

Description

Plot fixded coefficients of group-specific fixed effects model

Usage

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

Arguments

x	A cytoglmm 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))
group_fit <- CytoGLMM::cytogroup(df,
                                 protein_names = protein_names,
                                 condition = "condition",
                                 group = "donor")
plot(group_fit)
```

`plot_coeff` *Helper function to plot regression coeffcient*

Description

Helper function to plot regression coeffcient

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

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 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 *Extact and print bootstrap GLM fit*

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)
```

`print.cytoglmm` *Extact and print GLMM fit*

Description

Extact and print GLMM fit

Usage

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

Arguments

`x`	A `cytoglmm` 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))
glmm_fit <- CytoGLMM::cytoglmm(df,
                               protein_names = protein_names,
                               condition = "condition",
                               group = "donor")
print(glmm_fit)
```

remove_samples *Remove samples based on low cell counts*

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 *Extact and calculate p-values of bootstrap GLM fit*

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

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)
```

`summary.cytoglmm` *Extact and calculate p-values of GLMM fit*

Description

Extact and calculate p-values of GLMM fit

Usage

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

Arguments

`object`	A `cytoglmm` 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))
glmm_fit <- CytoGLMM::cytoglmm(df,
                               protein_names = protein_names,
                               condition = "condition",
                               group = "donor")
summary(glmm_fit)
```

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