

Package ‘protGear’

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Type Package

Title Protein Micro Array Data Management and Interactive Visualization

Version 1.2.0

Description A generic three-step pre-processing package for protein microarray data. This package contains different data pre-processing procedures to allow comparison of their performance. These steps are background correction, the coefficient of variation (CV) based filtering, batch correction and normalization.

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URL <https://github.com/Keniajin/protGear>

BugReports <https://github.com/Keniajin/protGear/issues>

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array_vars	<i>List the array structure variables</i>
------------	---

Description

A generic function returning a list with the data structure.

Usage

```
array_vars(
  channel = "635",
  totsamples,
  FG = "",
  BG = "",
  FBG = "",
  blockspersample,
  chip_path = "data/array_data",
  sampleID_path = "data/array_sampleID/",
  mig_prefix = "_first",
  machine = "",
  date_process = ""
)
```

Arguments

channel	A character indicating the channel that the data was scanned at. It is mostly included in the MFI variable names.
totsamples	A numeric value indicating the number of samples on a slide.
FG	Optional: A character indicating the name of the foreground variable name. if not specified its created as <code>paste0("F", channel, ".Median")</code>
BG	Optional: A character indicating the name of the background variable name. if not specified its created as <code>paste0("B", channel, ".Median")</code>
FBG	Optional: A character indicating the name of the foreground - background variable name. if not specified its created as <code>paste0("F", channel, ".Median...B", channel)</code>
blockspersample	A numeric value indicating the number of blocks in a mini-array. The ".gal" file can help in getting this
chip_path	A character indicating the path of the folder location with the array data.
sampleID_path	A character indicating the path of the folder location with the sample identifiers matching the array structure.
mig_prefix	Optional: A character indicating the identifier of an MIG dilution file
machine	Optional: A character indicating the machine used to process the data in the folder
date_process	Optional: A character indicating the date when the samples were processed.

Value

a list of parameters required to process the data
 genepix_vars

Examples

```
## specify the the parameters to process the data
genepix_vars <- array_vars(
## the channel the data was processed in
  channel = "635",
  ## folder where the array data is stored
  chip_path = "data/array_data",
  ## the number of samples per slide or in as single run
  totsamples = 21,
  ## How many blocks each sample occupies
  blockspersample = 2,
  ## folder where the array data samples id files are stored
  sampleID_path = "data/array_sampleID/",
  ## optional
  mig_prefix = "_first",
  machine = 1,
  date_process = "0520"
)
genepix_vars
```

best_CV_estimation *best CV estimation*

Description

A function to select the best CV by combining the replicates in duplicates. The function has been build for up to 3 replicates so far

Usage

```
best_CV_estimation(dataCV, slide_id, lab_replicates, cv_cut_off)
```

Arguments

dataCV	A data frame
slide_id	A character string containing the identifier of the data frame variable.
lab_replicates	A numeric value indicating the number of lab replicates.
cv_cut_off	a numeric value for the CV cut off. Should be between 0-100

Details

Select set of replicates with the best CV

Value

A data frame with the best CV's estimated

Examples

```
dataC <- readr::read_csv(system.file("extdata",
  "dataC.csv", package="protGear"))
## this file has 3 lab replicates and the default names
dataCV <- cv_estimation(dataC ,lab_replicates=3)
best_CV_estimation(dataCV,slide_id = "iden", lab_replicates = 3,
  cv_cut_off = 20)
```

bg_correct	<i>bg_correct</i>
------------	-------------------

Description

A generic function to perform background correction.

Usage

```
bg_correct(iden, Data1, genepix_vars, method = "subtract_local")
```

Arguments

iden	A character indicating the name of the object to be used under Data1
Data1	A data frame with sample identifiers merged with micro array data.
genepix_vars	A list of specific definitions of the experiment design. See array_vars .
method	a description of the background correction to be used. Possible values are "none", "subtract_local", "subtract_global", "movingmin_bg", "minimum_half", "edwards" or "normexp". The default is "subtract_local".

Details**Background correction**

The function implements background correction methods developed by [backgroundCorrect](#). But the `minimum_half` and `movingmin_bg` uses the block of the protein array as the grid. If `method="movingmin_bg"` the minimum background value within a block is subtracted. If `method="minimum_half"` then any intensity which is negative after background subtraction is reset to be equal to half the minimum positive value in a block. If `method="movingmin_value"` then any intensity which is negative after background subtraction is reset to the minimum positive value in a block. For `edwards` we implement a similar algorithm with `limma::backgroundCorrect(method="edwards")` and for 'normexp' we use the saddle-point approximation to maximum likelihood, [backgroundCorrect](#) for more details.

Value

A data frame with background corrected data

buffer_spots	<i>Extract buffer spots of data</i>
--------------	-------------------------------------

Description

A function to extract the buffer spots data. A buffer spot only has the solution for proprietary ingredients for stabilizing protein and minimizing evaporation.

Usage

```
buffer_spots(Data1, buffer_spot = "buffer")
```

Arguments

Data1	An object of the class data frame
buffer_spot	A character string containing the name of the buffer spots.

Value

A data frame of the buffer control spots

Examples

```
bg_correct_df <- readr::read_csv(system.file("extdata", "Data1_sample.csv",
package="protGear"))
buffer_spots(Data1 = bg_correct_df)
```

check_sampleID_files	<i>_End_Function_\\# Check existing sample ID names</i>
----------------------	---

Description

A generic function to check if the file(s) with the MFI values have a corresponding sample ID file. Sample ID file is a file with the identifiers for the samples in array file.

Usage

```
check_sampleID_files(genepix_vars)
```

Arguments

genepix_vars	A list of specific definitions of the experiment design. See array_vars .
--------------	---

Value

A file with missing corresponding sample ID files

Examples

```
genepix_vars <- array_vars(  
  channel = "635",  
  chip_path = system.file("extdata", "array_data/machine1/",  
    package="protGear"),  
  totsamples = 21,  
  blockspersample = 2,  
  mig_prefix = "_first",  
  machine = 1,  
  date_process = "0520"  
)  
check_sampleID_files(genepix_vars)
```

create_dir

Title Create directory function

Description

creating a directory

Usage

```
create_dir(path)
```

Arguments

path folder location to create a directory

Value

created directory

Examples

```
create_dir("data/sample_folder")
```

cv_by_sample_estimation

cv by sample

Description

A function to give the summary of the CV's by the sampleID

Usage

```
cv_by_sample_estimation(  
  dataCV,  
  cv_variable,  
  lab_replicates,  
  sampleID_var = "sampleID"  
)
```

Arguments

dataCV	A dataframe
cv_variable	A character string containing the identifier of the variable with CV values.
lab_replicates	A numeric value indicating the number of lab replicates.
sampleID_var	A character string containing the name of the sample identifier variable. Default set to 'sampleID'

Details

Summarise CV by samples

Value

A data frame of CV calculated by sample

Examples

```
dataC <- readr::read_csv(system.file("extdata",  
  "dataC.csv", package="protGear"))  
## this file has 3 lab replicates and the default names  
dataCV <- cv_estimation(dataC ,lab_replicates=3)  
cv_by_sample_estimation(dataCV, cv_variable = "cvCat_all",  
  lab_replicates = 3)
```

cv_estimation

cv_estimation

Description

A function to calculate the CV for the technical lab replicates. The default values are set as per the object names generated by machine

Usage

```
cv_estimation(  
  dataC,  
  lab_replicates,  
  sampleID_var = "sampleID",  
  antigen_var = "antigen",  
  replicate_var = "replicate",  
  mfi_var = "FMedianBG_correct",  
  cv_cut_off = 20  
)
```

Arguments

dataC	A dataset a data frame with feature variables to be used
lab_replicates	A numeric value indicating the number of lab replicates
sampleID_var	A character string containing the name of the sample identifier variable. Default set to 'sampleID'
antigen_var	A character string containing the name of the features/protein variable. Default to 'antigen'
replicate_var	A character string containing the name of the replicate variable. Default to 'replicate'
mfi_var	A character string containing the name of the variable with MFI value. Assuming background correction is done already. Default to 'FMedianBG_correct'
cv_cut_off	Optional value indicating the cut off of flagging CV's. Default set at 20.

Details

Coefficient of Variation

Value

A data frame where CV's of the replicates have been calculated

Examples

```
dataC <- readr::read_csv(system.file("extdata",  
  "dataC.csv", package="protGear"))  
## this file has 3 lab replicates and the default names  
cv_estimation(dataC ,lab_replicates=3)
```

extract_bg	<i>extract bg</i>
------------	-------------------

Description

A generic function to extract the background data for micro array data.

Usage

```
extract_bg(iden, data_files, genepix_vars = genepix_vars)
```

Arguments

iden	A character indicating the name of the object to be used under data_files.
data_files	A list of data objects with names utilised by iden.
genepix_vars	A list of specific definitions of the experiment design. See array_vars .

Details

Extract the background values

Value

A data frame of background values

Examples

```
## Not run:
genepix_vars <- array_vars(
  channel = "635",
  chip_path = system.file("extdata", "array_data/machine1/",
    package="protGear"),
  totsamples = 21,
  blockspersample = 2,
  mig_prefix = "_first",
  machine = 1,
  ## optional
  date_process = "0520"
)
#Define the data path
data_path <- paste0(genepix_vars$chip_path)
# List the file names to use
filenames <- list.files(genepix_vars$chip_path,
  pattern = '*.txt$|*.gpr$', full.names = FALSE
)
data_files <- purrr::map(
  .x = filenames,
  .f = read_array_files,
  data_path = data_path,
```

```

    genepix_vars = genepix_vars
  )
  data_files <- purrr::set_names(data_files,
  purrr::map(filenamees, name_of_files))
  names(data_files)
  extract_bg(iden ="KK2-06" , data_files=data_files,genepix_vars=genepix_vars)
  ## End(Not run)

```

launch_protGear_interactive
launch_protGear_interactive

Description

This is Function is to launch the shiny application

Usage

```
launch_protGear_interactive()
```

Value

launches the shiny interactive protGear app

Examples

```

app <- system.file("shiny-examples", "protGear_interactive",
"protGear_interactive.Rmd", package = "protGear")
if (app!=""){
  ## run this
  #launch_protGear_interactive()
}

```

launch_select *launch_select*

Description

This is Function is to launch mutiple shiny applications for protGear

Usage

```
launch_select(theApp)
```

Arguments

theApp accepts one of the folders containing the shiny application

Value

launches the app defined under theApp

Examples

```
validExamples <-
  list.files(system.file("shiny-examples", package = "protGear"))
#launch_select(validExamples[[1]])
```

matrix_normalise	<i>Normalize Arrays</i>
------------------	-------------------------

Description

Normalize Arrays

Usage

```
matrix_normalise(
  matrix_antigen,
  method = "log2",
  batch_correct = FALSE,
  batch_var1,
  batch_var2 = day_batches,
  return_plot = FALSE,
  plot_by_antigen = TRUE,
  control_antigens = NULL,
  array_matrix = NULL
)
```

Arguments

matrix_antigen	An object of class matrix with features/proteins as columns and samples as the rows
method	character string specifying the normalization method. Choices are "none", "log2", "vsn", "cyclic_loess", "cyclic_loess_log", "rlm"
batch_correct	A logical value indicating whether batch correction should be done or not
batch_var1	A character or factor vector of size similar to rows of matrix_antigen indicating the first batch.
batch_var2	A character or factor vector of size similar to rows of matrix_antigen indicating the second batch.
return_plot	A logical value indicating whether a plot is returned to show the results of normalisation.
plot_by_antigen	Logical to indicate whether to plot by antigen or not slide name for the matrix_antigen object.

control_antigens logical vector specifying the subset of spots which are non-differentially-expressed control spots, for use with method="rlm"

array_matrix An object of class dataframe or matrix used with method='rlm' indicating the sample index and

Value

A data frame of normalised values

Examples

```
matrix_antigen <- readr::read_csv(system.file("extdata",
"matrix_antigen.csv", package="protGear"))
#VSN
normlise_vsn <- matrix_normalise(as.matrix(matrix_antigen),
method = "vsn",
return_plot = TRUE
)
## log
normlise_log <- matrix_normalise(as.matrix(matrix_antigen),
method = "log2",
return_plot = TRUE
)
## cyclic_loess_log
normlise_cylic_log <- matrix_normalise(as.matrix(matrix_antigen),
method = "cyclic_loess_log",
return_plot = TRUE
)
```

merge_sampleID	<i>Merge sample ID with the array data</i>
----------------	--

Description

A generic function that merges the protein data with the sample identifiers or sample names. If the file does not have sample identifiers the function generates it automatically.

Usage

```
merge_sampleID(iden, data_files, genepix_vars, method)
```

Arguments

iden A character indicating the name of the object to be used under data_files.

data_files A list of data objects with names utilised by iden.

genepix_vars A list of specific definitions of the experiment design. See [array_vars](#).

method A description of the background correction to be used. See [bg_correct](#).

Value

a data frame merged with corresponding sample ID's. The sample ID are specified in the sample ID files

Examples

```
## Not run:
### Define the genepix_vars
genepix_vars <- array_vars(
  channel = "635",
  chip_path = system.file("extdata", "array_data/machine1/",
    package="protGear"),
  totsamples = 21,
  blockspersample = 2,
  mig_prefix = "_first",
  machine = 1,
  ## optional
  date_process = "0520"
)

## the path where the micro-array data is located
data_path <- paste0(genepix_vars$chip_path)
filenames <- list.files(genepix_vars$chip_path,
  pattern = "*.txt$|*.gpr$", full.names = FALSE
)
## create a list of all the files
data_files <- purrr::map(
  .x = filenames,
  .f = read_array_files,
  data_path = data_path,
  genepix_vars = genepix_vars
)
data_files <- purrr::set_names(data_files,
  purrr::map(filenames, name_of_files))
## merge the lab data with samples and perform bg correction
merge_sampleID(iden = "KK2-06", data_files = data_files,
  genepix_vars =genepix_vars,method = "subtract_global" )
## End(Not run)
```

minpositive

Get the minimum positive value

Description

Get the minimum positive value

Usage

```
minpositive(x)
```

Arguments

x A numeric vector or variable

Value

Returns the minimum positive value in an object

Examples

```
minpositive(c(-1,-2,3,5,6,7,8,9,10))
```

name_of_files	<i>Object names of a list</i>
---------------	-------------------------------

Description

A generic function returning a vector with the names of files in the same directory. Removes the file extension

Usage

```
name_of_files(i)
```

Arguments

i - a list filenames with .txt or .gpr extension

Value

a list of file names

name

Examples

```
name_of_files("KK2-06.txt")
```

output_trend_stats	<i>Trend test using Cox–Stuart (C–S) and Mann–Kendall (M–K) trend tests</i>
--------------------	---

Description

Trend test using Cox–Stuart (C–S) and Mann–Kendall (M–K) trend tests

Usage

```
output_trend_stats(name, p_val, z_val)
```

Arguments

name	Name of the test
p_val	p value from the test
z_val	the Z value of the test

Value

A statistics of mean standard deviation trend

Examples

```
output_trend_stats(name="t.test",p_val=0.001, z_val=5)
```

plot_bg	<i>Plot background</i>
---------	------------------------

Description

A generic function for plotting of R objects.

Usage

```
plot_bg(df, x_axis = "antigen", bg_MFI = "BG_Median", log_mfi = TRUE)
```

Arguments

df	A default dataset to use for plot.
x_axis	The variable on the x axis
bg_MFI	A numeric variable describing which is the background MFI
log_mfi	a logical value indicating whether the MFI values should be log transformed or not.

Value

A ggplot of background values

Examples

```
## Not run:
#After extracting the background using \code{\link{extract_bg}}
#we plot the data using
allData_bg <- readr::read_csv(system.file("extdata", "bg_example.csv",
  package="protGear"))
plot_bg(allData_bg,
  x_axis = "antigen",
  bg_MFI = "BG_Median", log_mfi = TRUE
)
## End(Not run)
```

plot_buffer	<i>Plot the buffer values</i>
-------------	-------------------------------

Description

Plot the buffer values

Usage

```
plot_buffer(
  df = buffers,
  buffer_names = "antigen",
  buffer_mfi = "FMedianBG_correct",
  slide_id = ".id"
)
```

Arguments

df	A data frame to be used to plot
buffer_names	A character string containing the name of the variable with buffer spots. Default set to 'antigen'.
buffer_mfi	A character string containing the name of the variable with MFI value. Assuming background correction is done already. Default to 'FMedianBG_correct'
slide_id	A character string containing the name of the slide/array identifier variable.

Value

plot of buffer spots

Examples

```
buffers <- readr::read_csv(system.file("extdata", "buffers_sample2.csv",  
package="protGear"))  
plot_buffer(df=buffers,buffer_names = "sampleID")
```

plot_FB

plot_FB

Description

A generic function for plotting the background and foreground values.

Usage

```
plot_FB(  
  df,  
  antigen_name = "antigen",  
  bg_MFI = "BG_Median",  
  FG_MFI = "FBG_Median",  
  log_mfi = FALSE  
)
```

Arguments

df	An object containing the data to which the plot is done.
antigen_name	The variable describing which features/proteins/ antibodies in the data should be used to plot
bg_MFI	A numeric variable describing which is the background MFI
FG_MFI	A numeric variable describing which is the foreground MFI
log_mfi	a logical value indicating whether the MFI values should be log transformed or not.

Details

Plot foreground and background values

Value

a ggplot of foreground vs background MFI values

Examples

```
## Not run:
#After extracting the background using \code{\link{extract_bg}}
#we plot the data using
allData_bg <- readr::read_csv(system.file("extdata",
"bg_example.csv", package="protGear"))
plot_FB(allData_bg,
antigen_name = "antigen",
bg_MFI = "BG_Median", FG_MFI = "FBG_Median", log = FALSE
)
## End(Not run)
```

plot_normalised	<i>Comparison of normalised data by sample</i>
-----------------	--

Description

Comparison of normalised data by sample

Usage

```
plot_normalised(exprs_normalised_df, method, batch_correct)
```

Arguments

```
exprs_normalised_df
    a normalised data frame

method
    the method of normalisation used

batch_correct
    the batch correction
```

Value

A ggplot of normalised data

Examples

```
matrix_antigen <- readr::read_csv(system.file("extdata",
"matrix_antigen.csv", package="protGear"))
normlise_vsn <- matrix_normalise(as.matrix(matrix_antigen),
method = "vsn",
return_plot = FALSE
)
plot_normalised(normlise_vsn,method="vsn",batch_correct=FALSE)
```

plot_normalised_antigen

Comparison of normalised data by feature

Description

Comparison of normalised data by feature

Usage

```
plot_normalised_antigen(exprs_normalised_df, method, batch_correct)
```

Arguments

exprs_normalised_df
a normalised data frame

method
the method of normalisation used

batch_correct
the batch correction

Value

A ggplot of various normalisation approaches

Examples

```
matrix_antigen <- readr::read_csv(system.file("extdata",  
"matrix_antigen.csv", package="protGear"))  
normlise_vsn <- matrix_normalise(as.matrix(matrix_antigen),  
method = "vsn",  
return_plot = FALSE  
)  
plot_normalised_antigen(normlise_vsn,method="vsn",batch_correct=FALSE)
```

read_array_files

Read array files

Description

This helps to read the chip file(s).

Usage

```
read_array_files(i, data_path, genepix_vars)
```

Arguments

i	The name of the file which the data are to be read from.
data_path	The path where the file with the data is located
genepix_vars	A list of specific definitions of the experiment design. See array_vars .

Details

Read multiple array files

Value

a number of data frames in the global environment

Examples

```
## Not run:
genepix_vars <- array_vars(
  channel = "635",
  chip_path = system.file("extdata", "array_data/machine1/",
  package="protGear"),
  totsamples = 21,
  blockspersample = 2,
  mig_prefix = "_first",
  machine = 1,
  date_process = "0520"
)
file_read <- "KK2-06.txt"
read_array_files(i=file_read,
  data_path=system.file("extdata", "array_data/machine1/",
  package="protGear"), genepix_vars=genepix_vars)
## End(Not run)
```

read_array_visualize *Read a gpr file to visualize*

Description

Read a gpr file to visualize

Usage

```
read_array_visualize(infile)
```

Arguments

infile a .gpr file to be used to visualize the expression intensities of the slide spots

Value

a data frame to visualize the background or foreground values

Examples

```
## Not run:  
read_array_visualize(infile = system.file("extdata",  
"/array_data/machine1/KK2-06.txt", package="protGear"))  
## End(Not run)
```

rlm_normalise_matrix *Nomrmalise using RLM*

Description

A function for method='rlm' from [matrix_normalise](#).

Usage

```
rlm_normalise_matrix(matrix_antigen, array_matrix, control_antigens)
```

Arguments

matrix_antigen A matrix with antigen data
array_matrix A matrix with control antigen data
control_antigens
the control antigens for RLM normalisation

Value

A RLM normalised data frame

Examples

```
matrix_antigen <- readr::read_csv(system.file("extdata",  
"matrix_antigen.csv", package="protGear"))  
# rlm_normalise_matrix(matrix_antigen=matrix_antigen,  
# array_matrix=array_matrix,  
# control_antigens=control_antigens)
```

tag_subtract	<i>tag_subtract</i>
--------------	---------------------

Description

_End_Function_\\#

Usage

```
tag_subtract(
  dataC_mfi,
  tag_antigens,
  mean_best_CV_var,
  tag_file,
  batch_vars,
  sampleID_var = "sampleID",
  antigen_var = "antigen"
)
```

Arguments

dataC_mfi	A dataframe
tag_antigens	A character vector with the names of proteins or antigens used as TAG.
mean_best_CV_var	A character string containing the identifier of the variable with the MFI values.
tag_file	A data frame with variables antigen, TAG, TAG_name to show the TAG for the different antigens or proteins in dataC_mfi
batch_vars	A list of characters identifying variables in dataC_mfi for indicating batch.
sampleID_var	A character string containing the name of the sample identifier variable. Default set to 'sampleID'
antigen_var	A character string containing the name of the features/protein variable. Default to 'antigen'

Details

Subtract the purification TAG data

Value

A data frame of TAG values subtracted

Examples

```

tag_file <- readr::read_csv(system.file("extdata", "TAG_antigens.csv",
package="protGear"))
tag_antigens <- c("CD4TAG", "GST", "MBP")
batch_vars <- list(machine = "m1", day = "0520")
dataC <- readr::read_csv(system.file("extdata", "dataC.csv",
package="protGear"))
## this file has 3 lab replicates and the default names
dataCV <- cv_estimation(dataC ,lab_replicates=3)
dataCV_best2 <- best_CV_estimation(dataCV,slide_id = "iden",
lab_replicates = 3, cv_cut_off = 20)
tag_subtract(dataCV_best2,tag_antigens=tag_antigens,
mean_best_CV_var="mean_best_CV",
tag_file = tag_file,antigen_var = "antigen", batch_vars = batch_vars)

```

visualize_slide

Visualize the slide mimicking the original scan image.

Description

Visualize the slide mimicking the original scan image.

Usage

```
visualize_slide(infile, MFI_var, interactive = FALSE, d_f = NA)
```

Arguments

infile	a .gpr file to be used to visualize the expression intensities of the slide spots
MFI_var	the MFI variable to plot, can be either the background or foreground value
interactive	a logical to specify whether an interactive graph is returned or not
d_f	a data frame with array data

Value

A ggplot of slide foreground values

Examples

```

## Not run:
visualize_slide(
infile = system.file("extdata", "/array_data/machine1/KK2-06.txt",
package="protGear"),
MFI_var = "B635 Median"
)
## End(Not run)

```

visualize_slide_2d	<i>Visualize the slide mimicking the original scan image using a 2d plot.</i>
--------------------	---

Description

Visualize the slide mimicking the original scan image using a 2d plot.

Usage

```
visualize_slide_2d(infile, MFI_var, d_f = NA)
```

Arguments

<code>infile</code>	- a .gpr file to be used to visualize the expression intensities of the slide spots
<code>MFI_var</code>	the MFI variable to plot, can be either the background or foreground value
<code>d_f</code>	a data frame with array data

Value

A 2d plot of either the background or foreground values

Examples

```
## Not run:  
visualize_slide_2d(  
  infile = system.file("extdata", "/array_data/machine1/KK2-06.txt",  
    package="protGear"),  
  MFI_var = "B635 Median"  
)  
## End(Not run)
```

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