

Package ‘ProteoMM’

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Title Multi-Dataset Model-based Differential Expression Proteomics Analysis Platform

Version 1.27.0

Description ProteoMM is a statistical method to perform model-based peptide-level differential expression analysis of single or multiple datasets. For multiple datasets ProteoMM produces a single fold change and p-value for each protein across multiple datasets.

ProteoMM provides functionality for normalization, missing value imputation and differential expression.

Model-based peptide-level imputation and differential expression analysis component of package follows the analysis described in “A statistical framework for protein quantitation in bottom-up MS based proteomics” (Karpievitch et al. Bioinformatics 2009).

EigenMS normalisation is implemented as described in "Normalization of peak intensities in bottom-up MS-based proteomics using singular value decomposition."
(Karpievitch et al. Bioinformatics 2009).

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LazyData TRUE

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convert_log2	<i>Convert values in a matrix to log2 transformed values</i>
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Description

convert_log2 replaces 0's with NA's than does a log2 transformation Replacing 0's with NA's is the correct approach to Proteomics data analysis as 0's are not values that should be left in the data where no observation was made, see citation below. Karpievitch et al. 2009 "Normalization of peak intensities in bottom-up MS-based proteomics using singular value decomposition". PMID: 19602524 Karpievitch et al. 2009 "A statistical framework for protein quantitation in bottom-up MS-based proteomics". PMID: 19535538

Usage

```
convert_log2(mm, use_cols)
```

Arguments

mm	a dataframe of raw intensities in format: (# peptides)x(# samples+possibly peptide & protein information (metadata))
use_cols	vector of column indecies that make up the intensities usually in sequential order but do not have to be user is responsible for making sure that specified columns are indeed numeric and correspond to intensities for each sample

Value

matrix of log2 transformed intensities where 0's were replaced with NA's prior to transformation

Examples

```
data(mm_peptides)
head(mm_peptides)
intsCols = 8:13
metaCols = 1:7
m_logInts = make_intencities(mm_peptides, intsCols)
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts) # 0's replaced with NAs and
                                     # log2 transform applied
```

eigen_pi	<i>Compute PI - proportion of observations missing completely at random</i>
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Description

Compute PI - proportion of observations missing completely at random

Usage

```
eigen_pi(m, topplot = TRUE)
```

Arguments

m	matrix of abundances, numsmamples x numpeptides
topplot	TRUE/FALSE plot mean vs proportion missing curve and PI

Value

pi estimate of the proportion of observations missing completely at random

Contributed by Shelley Herbrich & Tom Taverner for Karpievitch et al. 2009

Examples

```
data(mm_peptides)
intsCols = 8:13
metaCols = 1:7
m_logInts = make_intencities(mm_peptides, intsCols)
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
my.pi = eigen_pi(m_logInts, topplot=TRUE)
```

eig_norm1

Identify bias trends

Description

First portion of EigenMS: Identify eigentrends attributable to bias, allow the user to adjust the number (with caution! if desired) before normalizing with eig_norm2. Ref: "Normalization of peak intensities in bottom-up MS-based proteomics using singular value decomposition" Karpievitch YV, Taverner T, et al. 2009, Bioinformatics Ref: "Metabolomics data normalization with EigenMS" Karpievitch YK, Nikolic SB, Wilson R, Sharman JE, Edwards LM 2014, PLoS ONE

Usage

```
eig_norm1(m, treatment, prot.info, write_to_file = "")
```

Arguments

m	number of peptides x number of samples matrix of log-transformed expression data, metadata not included in this matrix
treatment	either a single factor indicating the treatment group of each sample i.e. [1 1 1 1 2 2 2...] or a data frame of factors, eg: treatment= data.frame(cbind(data.frame(Group), data.frame(Time)))
prot.info	2+ colum data frame, pepID, prID columns IN THAT ORDER. IMPORTANT: pepIDs must be unique identifiers and will be used as Row Names If normalizing non-proteomics data, create a column such as: paste('ID_',seq_len(num_rows), sep='') Same can be dome for ProtIDs, these are not used for normalization but are kept for future analyses
write_to_file	if a string is passed in, 'complete' peptides (peptides with NO missing observations) will be written to that file name

Value

A structure with multiple components

m, treatment, prot.info, grp initial parameters passed into the function, returned for future reference

my.svd matrices produced by SVD

pres matrix of peptides that can be normalized, i.e. have enough observations for ANOVA

n.treatment number of factors passed in

n.u.treatment number of unique treatment facotr combinations, eg: Factor A: a a a c c c c Factor B: 1 1 2 2 1 1 2 2 then: n.treatment = 2; n.u.treatment = 4

h.c number of bias trends identified

present names/IDs of peptides in variable 'pres'

complete complete peptides with no missing values, these were used to compute SVD

toplot1 trends automatically identified in raw data, can be plotted at a later time

Tk scores for each bias trend, eigenvalues

ncompl number of complete peptides with no missing observations

Examples

```
data(mm_peptides)
head(mm_peptides)
# different from parameter names as R uses outer name spaces
# if variable is undefined
intsCols = 8:13
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(mm_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
# 3 samples for CG and 3 for mCG
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))

# ATTENTION: SET RANDOM NUMBER GENERATOR SEED FOR REPRODUCIBILITY !!
set.seed(123) # Bias trends are determined via a permutaion, results may
# vary slightly if a different seed is used, such as when set.seed()
# function is not used

mm_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)
```

eig_norm2

EigenMS normalization

Description

Eliminate the effects of systematic bias identified in eig_norm1() Ref: "Normalization of peak intensities in bottom-up MS-based proteomics using singular value decomposition" Karpievitch YV, Taverner T et al. 2009, Bioinformatics Ref: "Metabolomics data normalization with EigenMS" Karpievitch YK, Nikolic SB, Wilson R, Sharman JE, Edwards LM Submitted to PLoS ONE.

Usage

```
eig_norm2(rv)
```

Arguments

rv return value from the eig_norm1 if user wants to change the number of bias trends that will be eliminated h.c in rv should be updates to the desired number

Value

A structure with multiple components

normalized matrix of normalized abundances with 2 columns of protein and peptide names

norm_m matrix of normalized abundances, no extra columns

eigentrends trends found in raw data, bias trends up to h.c

norm.svd trends in normalized data, if one wanted to plot at later time

exPeps peptides excluded due to not enough peptides or exception in fitting a linear model

Examples

```
data(mm_peptides)
head(mm_peptides)
# different from parameter names as R uses outer name
# spaces if variable is undefined
intsCols = 8:13
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(mm_peptides, intsCols)
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))

set.seed(123) # set for reproducibility of eig_norm1
mm_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)
```

g.test

G Test for presence - absence analysis

Description

Log-likelihood test for independence & goodness of fit. `g.test()` performs Williams' and Yates' correction; Monte Carlo simulation of p-values, via `gtestsim.c`. MC requires recompilation of R. Written by Peter Hurd (V3.3 Pete Hurd Sept 29 2001, phurd AT ualberta.ca). Yuliya Karpievitch added comments for ease of understanding and incorporated into ProteoMM. G & q calculation from Sokal & Rohlf (1995) Biometry 3rd ed., TOI Yates correction taken from Mike Camanns 2x2 G-test function, GOF Yates correction as described in Zar (2000), more stuff taken from `cstest's` `chisq.test()`.

Usage

```
g.test(x, y = NULL, correct = "none", p = rep(1/length(x),
length(x)))
```

Arguments

x vector of boolean values corresponding to presence & absence eg: `c(TRUE, TRUE, FALSE, FALSE)` for present present absent absent values. Order of TRUE/FALSE does not matter, can be used interchangeably. Same length as parameter `y`

y	vector treatments (factor) corresponding to values in x, same length as x eg: <code>as.factor(c('grp1', 'grp1', 'grp2', 'grp2'))</code>
correct	correction to apply, options: "yates", "williams", "none" default: "none" NOTE: in ProteoMM we only tested & used correction = "none"
p	default: <code>rep(1/length(x), length(x))</code> , used in Yates correction NOTE: in ProteoMM we only tested & used the default parameter value

Value

htest object the following variables

statistic value of the G statistic produced by g test

parameter degrees of freedom of the test

p.value p-value

method method used to produce statistic and p-value

data.name data passed in to the function

observed matrix of observed counts

expected matrix of expected counts

Examples

```
g.test(c(TRUE, TRUE, FALSE, FALSE),
       as.factor(c('grp1', 'grp1', 'grp2', 'grp2')))
```

get_presAbs_prots	<i>Get Presence/Absence Proteins</i>
-------------------	--------------------------------------

Description

Function `get_presAbs_prots()` produces a subset of protein meta data and intensities for multiple datasets pass in as a list. If a single dataset is passed in (list of length one) it will be processed in the same way as longer lists.

Usage

```
get_presAbs_prots(mm_list, prot.info, protnames_norm, prot_col_name)
```

Arguments

mm_list	list of matrices of intensities for each experiment. Dimentions: numpeptides x numsamples different for each dataset.
prot.info	list of protein and peptide metadata/mappings for each matrix in mm_list, data.frames "parallel" to matrices in mm_list.
protnames_norm	list of protein pidentifies to be used to determine peptides that will be placed into Presence/Absence analysis category due to too many missing peptides. Taken from the return value from <code>eig_norm2()</code> .
prot_col_name	column name (string) that will be used to get ProteinIDs in the raw data matrices

Value

list of lists of length 2

intensities list of intensities in the same order and of the same length as the number of datasets that were passed into the function

protein metadata list of protein metadata in the same order and of the same length as the number of datasets that as were passed into the function

Examples

```
# Load mouse dataset
data(mm_peptides)
head(mm_peptides)
intsCols = 8:13
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(mm_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG', 'CG', 'CG', 'mCG', 'mCG', 'mCG'))
mm_m_ints_eig1 = eig_norm1(m=m_logInts, treatment=grps, prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)

# Load human dataset
data(hs_peptides)
head(hs_peptides)
intsCols = 8:13
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(hs_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(hs_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG', 'CG', 'CG', 'mCG', 'mCG', 'mCG'))
hs_m_ints_eig1 = eig_norm1(m=m_logInts, treatment=grps, prot.info=m_prot.info)
hs_m_ints_eig1$h.c # check the number of bias trends detected
hs_m_ints_norm = eig_norm2(rv=hs_m_ints_eig1)

# Set up for presence/absence analysis
raw_list = list()
norm_imp_prot.info_list = list()
raw_list[[1]] = mm_m_ints_eig1$m
raw_list[[2]] = hs_m_ints_eig1$m
norm_imp_prot.info_list[[1]] = mm_m_ints_eig1$prot.info
norm_imp_prot.info_list[[2]] = hs_m_ints_eig1$prot.info

protnames_norm_list = list()
protnames_norm_list[[1]] = unique(mm_m_ints_norm$normalized$MatchedID)
protnames_norm_list[[2]] = unique(hs_m_ints_norm$normalized$MatchedID)

presAbs_dd = get_presAbs_prots(mm_list=raw_list,
                               prot.info=norm_imp_prot.info_list,
                               protnames_norm=protnames_norm_list,
                               prot_col_name=2)
```

hs_peptides

hs_peptides - peptide-level intensities for human

Description

A dataset containing the protein and peptide information and peptide-level intensities for 6 samples: 3 CG and 3 mCG groups. There are 69 proteins. The columns are as follows:

Usage

```
data(hs_peptides)
```

Format

A data frame with 695 rows and 13 columns, comprising 7 columns of metadata and 6 columns of peptide intensities. 69 proteins.

Details

- Sequence - peptide sequence - randomly chosen from a larger list of sequences
- MatchedID - numeric ID that links proteins in the two datasets, unnecessary if datasets are for the same species
- ProtID - protein ID, artificial protein ID, eg. Prot1, Prot2, ...
- GeneID - gene ID, artificial gene ID, eg. Gene1, Gene2, ...
- ProtName - artificial Protein Name
- ProtIDLong - long protein ID, full protein name, here artificially simulated
- GeneIDLong - long gene ID, full gene name, here artificially simulated
- CG1 - raw intensity column for sample 1 in CG group
- CG2 - raw intensity column for sample 2 in CG group
- CG3 - raw intensity column for sample 3 in CG group
- mCG1 - raw intensity column for sample 1 in mCG group
- mCG2 - raw intensity column for sample 2 in mCG group
- mCG3 - raw intensity column for sample 3 in mCG group

makeLMFormula

String linear model formula suitable

Description

Makes a string linear model formula suitable for the right hand side of the equation passed into lm()

Usage

```
makeLMFormula(eff, var_name = "")
```

Arguments

eff treatment group ordering for all samples being analysed. Single factor with 2+ treatment groups. Used to generate formula and contrasts for `lm()`.

var_name string variable name to use in the formula

Details

eig_norm1 and **eig_norm2** Here we incorporate the model matrix from EigenMS normalization to find the significant trends in the matrix of residuals.

Value

data structure with linea model formula and contrasts

lm.formula Lienar model formula suitable for right hand side of ' ~' in `lm()`, ~ is not included in the formula

lm.params contrasts for `lm()`, here sum-to-zero constraint only

Examples

```
grps = as.factor(c('CG', 'CG', 'CG', 'mCG', 'mCG', 'mCG'))
makeLMFormula(grps, 'TREATS')
```

make_intencities	<i>Subdivide data into intensities columns only</i>
------------------	---

Description

Subdivide a data frame of protein intensities and metadata into intensities only. No row names will be provided.

Usage

```
make_intencities(mm, use_cols)
```

Arguments

mm data frame of metadata and intensities as a single data frame

use_cols column numbers to subset and return, no range checking no range checking on the column indices is performed

Value

m_ints data frame of intensities only

Examples

```
data(mm_peptides)
head(mm_peptides)
intsCols = 8:13 # different from parameter names as R uses outer name
               # spaces if variable is undefined
m_logInts = make_intencities(mm_peptides, intsCols)
```

make_meta	<i>Subdivide data into metadata columns only</i>
-----------	--

Description

Subdivide a data frame of protein metadata and intensities into a data frame of meta data only

Usage

```
make_meta(mm, use_cols)
```

Arguments

mm	data frame of metadata and intensities as a single data frame
use_cols	column numbers to subset and return, no range checking on the column indices is performed

Value

m_ints data frame of intensities only

Examples

```
data(mm_peptides)
head(mm_peptides)
metaCols = 1:7 # reusing this variable
m_prot.info = make_meta(mm_peptides, metaCols)
```

MBimpute	<i>Model-Based Imputation of missing values</i>
----------	---

Description

Impute missing values based on information from multiple peptides within a protein. Expects the data to be filtered to contain at least one observation per treatment group. For experiments with lower overall abundances such as multiplexed experiments check if the imputed value is below 0, if so value is reimputed until it is above 0.

Usage

```
MBimpute(mm, treatment, prot.info, pr_ppos = 2, my.pi = 0.05,
  compute_pi = FALSE)
```

Arguments

<code>mm</code>	number of peptides x number of samples matrix of intensities
<code>treatment</code>	vector indicating the treatment group of each sample eg <code>as.factor(c('CG','CG','CG','mCG','mCG','mCG'))</code> or <code>c(1,1,1,1,2,2,2,2)</code>
<code>prot.info</code>	protein metadata, 2+ columns: peptide IDs, protein IDs, etc
<code>pr_ppos</code>	column index for protein ID in <code>prot.info</code>
<code>my.pi</code>	PI value, estimate of the proportion of peptides missing completely at random, as compared to censored at lower abundance levels default values of 0.05 is usually reasonable for missing completely at random values in proteomics data
<code>compute_pi</code>	TRUE/FALSE (default=FALSE) estimate Pi is set to TRUE, otherwise use the provided value. We consider $Pi=0.05$ a reasonable estimate for observations missing completely at random in proteomics experiments. Thus values is set to NOT estimate Pi by default. Note: spline smoothing can sometimes produce values of Pi outside the range of possible values.

Value

A structure with multiple components

y_imputed number of peptides x m matrix of peptides with no missing data

imp_prot.info imputed protein info, 2+ columns: peptide ID, protein IDs, etc Dimensions should be the same as passed in

Examples

```
data(mm_peptides)
head(mm_peptides)
intsCols = 8:13 # different from parameter names as R uses outer name spaces
                # if variable is undefined
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(mm_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG','CG','CG','mCG','mCG','mCG'))

set.seed(135)
mm_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)
mm_prot.info = mm_m_ints_norm$normalized[,1:7]
mm_norm_m = mm_m_ints_norm$normalized[,8:13]

# ATTENTION: SET RANDOM NUMBER GENERATOR SEED FOR REPRODUCIBILITY !!
set.seed(125) # if nto set every time results will be different
imp_mm = MBimpute(mm_norm_m, grps, prot.info=mm_prot.info, pr_ppos=2,
                  my.pi=0.05, compute_pi=FALSE)
```

mm_peptides

*mm_peptides - peptide-level intensities for mouse***Description**

A dataset containing the protein and peptide information and peptide-level intensities for 6 samples: 3 CG and 3 mCG groups. There are 69 proteins. The columns are as follows:

Usage

```
data(mm_peptides)
```

Format

A data frame with 1102 rows and 13 columns, comprising 7 columns of metadata and 6 columns of peptide intensities. 69 proteins.

Details

- Sequence - peptide sequence - randomly chosen from a larger list of sequences
- MatchedID - numeric ID that links proteins in the two datasets, unnecessary if datasets are for the same species
- ProtID - protein ID, artificial protein ID, eg. Prot1, Prot2, ...
- GeneID - gene ID, artificial gene ID, eg. Gene1, Gene2, ...
- ProtName - artificial Protein Name
- ProtIDLong - long protein ID, full protein name, here artificially simulated
- GeneIDLong - long gene ID, full gene name, here artificially simulated
- CG1 - raw intensity column for sample 1 in CG group
- CG2 - raw intensity column for sample 2 in CG group
- CG3 - raw intensity column for sample 3 in CG group
- mCG1 - raw intensity column for sample 1 in mCG group
- mCG2 - raw intensity column for sample 2 in mCG group
- mCG3 - raw intensity column for sample 3 in mCG group

peptideLevel_DE

*Model-Based differential expression analysis***Description**

Model-Based differential expression analysis is performed on peptide level as described in Karpievitch et al. 2009 "A statistical framework for protein quantitation in bottom-up MS-based proteomics" Bioinformatics.

Usage

```
peptideLevel_DE(mm, treatment, prot.info, pr_ppos = 2)
```

Arguments

<code>mm</code>	m x n matrix of intensities, num peptides x num samples
<code>treatment</code>	vector indicating the treatment group of each sample ie [1 1 1 1 2 2 2 2...]
<code>prot.info</code>	2+ column data frame of peptide ID, protein ID, etc. columns
<code>pr_ppos</code>	- column index for protein ID in prot.info. Can restrict to be #2...

Value

A data frame with the following columns:

ProtID protein identification information taken from prot.info, 1 column used to identify proteins

FC fold change

p-value p-value for the comparison between 2 groups (2 groups only here)

BH-adjusted p-value Benjamini-Hochberg adjusted p-values

Examples

```
data(mm_peptides)
head(mm_peptides)
# different from parameter names as R uses outer
# name spaces if variable is undefined
intsCols = 8:13
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(mm_peptides, intsCols)
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))

set.seed(135) # results rarely vary due to the random seed for EigenMS
mm_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)
mm_prot.info = mm_m_ints_norm$normalized[,1:7]
mm_norm_m = mm_m_ints_norm$normalized[,8:13]

set.seed(131) # important to reproduce the results later
imp_mm = MBimpute(mm_norm_m, grps, prot.info=mm_prot.info,
                  pr_ppos=2, my.pi=0.05,
                  compute_pi=FALSE)
DE_res = peptideLevel_DE(imp_mm$y_imputed,
                        grps, mm_m_ints_norm$normalized[,metaCols],
                        pr_ppos=2)
```

peptideLevel_PresAbsDE

Presence/Absence peptide-level analysis

Description

Presence/Absence peptide-level analysis uses all peptides for a protein as IID to produce 1 p-value across multiple (2+) datasets. Significance is estimated using a g-test which is suitable for two treatment groups only.

Usage

```
peptideLevel_PresAbsDE(mm, treatment, prot.info, pr_ppos = 2)
```

Arguments

<code>mm</code>	<code>m x n</code> matrix of intensities, num peptides x num samples
<code>treatment</code>	vector indicating the treatment group of each sample ie [1 1 1 1 2 2 2 2...]
<code>prot.info</code>	2+ column data frame of peptide ID, protein ID, etc columns
<code>pr_ppos</code>	- column index for protein ID in <code>prot.info</code> . Can restrict to be #2...

Value

A list of length two items:

ProtIDused protein identification information taken from `prot.info`, a column used to identify proteins

FC Approximation of the fold change computed as percent missing observations group 1 minus in percent missing observations group 2

P_val p-value for the comparison between 2 groups (2 groups only here)

BH_P_val Benjamini-Hochberg adjusted p-values

statistic statistic returned by the g-test, not very useful as depends on the direction of the test and can produce all 0's

num_peptides number of peptides within a protein

metadata all columns of metadata from the passed in matrix

Examples

```
# Load mouse dataset
data(mm_peptides)
head(mm_peptides)
intsCols = 8:13 # different from parameter names as R uses
                # outer name spaces if variable is undefined
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(mm_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))

set.seed(135)
mm_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)

# Load human dataset
data(hs_peptides)
head(hs_peptides)
intsCols = 8:13 # different from parameter names as R
                # uses outer name spaces if variable is undefined
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(hs_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(hs_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
```

```

grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))

set.seed(137) # different seed for different organism
hs_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
hs_m_ints_eig1$h.c # check the number of bias trends detected
hs_m_ints_norm = eig_norm2(rv=hs_m_ints_eig1)

# Set up for presence/absence analysis
raw_list = list()
norm_imp_prot.info_list = list()
raw_list[[1]] = mm_m_ints_eig1$m
raw_list[[2]] = hs_m_ints_eig1$m
norm_imp_prot.info_list[[1]] = mm_m_ints_eig1$prot.info
norm_imp_prot.info_list[[2]] = hs_m_ints_eig1$prot.info

protnames_norm_list = list()
protnames_norm_list[[1]] = unique(mm_m_ints_norm$normalized$MatchedID)
protnames_norm_list[[2]] = unique(hs_m_ints_norm$normalized$MatchedID)

presAbs_dd = get_presAbs_protos(mm_list=raw_list,
                                prot.info=norm_imp_prot.info_list,
                                protnames_norm=protnames_norm_list,
                                prot_col_name=2)

presAbs_de = peptideLevel_PresAbsDE(presAbs_dd[[1]][[1]],
                                     grps, presAbs_dd[[2]][[1]],
                                     pr_ppos=2)

```

plot_1prot

Plot trends for a single protien

Description

Plot peptide trends for a protein

Usage

```
plot_1prot(mm, prot.info, prot_to_plot, prot_to_plot_col, gene_name,
           gene_name_col, colors, mylabs)
```

Arguments

mm	matrix of raw intensities
prot.info	metadata for the intensities in mm
prot_to_plot	protein ID to plot
prot_to_plot_col	protein ID column index
gene_name	gene ID to plot
gene_name_col	gene ID to plot column index
colors	what colors to plot peptide abundances as, most commonly should be treatment groups
mylabs	sample labels to be plotted on x-axis

Value

Nil

plot_3_pep_trends_NOfile

Plot peptide trends

Description

Plot Raw, Normalized and Normalized & Imputed peptide trends for a protein

Usage

```
plot_3_pep_trends_NOfile(mm, prot.info, sorted_norm_m, sorted_prot.info,
  imp_mm, imp_prot.info, prot_to_plot, prot_to_plot_col, gene_name,
  gene_name_col, mylabs)
```

Arguments

mm	matrix of raw intensities
prot.info	metadata for the intensities in mm
sorted_norm_m	normalized intensities, possibly fewer than in mm due to filtering out peptides with fewer than one observation per treatment group
sorted_prot.info	metadata for the intensities in sorted_norm_m
imp_mm	imputed intensities (post normalization)
imp_prot.info	metadata for the imputed intensities in imp_mm
prot_to_plot	protein ID to plot
prot_to_plot_col	protein ID column index
gene_name	gene ID to plot
gene_name_col	gene ID to plot column index
mylabs	sample labels to be plotted on x-axis

Value

Nil

Examples

```
data("hs_peptides") # loads variable hs_peptides
intsCols = 8:13 # column indeces that contain intensities
m_logInts = make_intencities(hs_peptides, intsCols)
# replace 0's with NA's as NA's are more appropriate
# for anlysis and log2 transform
m_logInts = convert_log2(m_logInts)
# column indices that contain metadata such as protein IDs and sequences
metaCols = 1:7
m_prot.info = make_meta(hs_peptides, metaCols)
```

```

grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))

set.seed(135)
hs_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
hs_m_ints_eig1$h.c = 2 # looks like there are 2 bias trends at least
hs_m_ints_norm = eig_norm2(rv=hs_m_ints_eig1)
hs_prot.info = hs_m_ints_norm$normalized[,metaCols]
hs_norm_m = hs_m_ints_norm$normalized[,intsCols]

set.seed(125)
imp_hs = MBimpute(hs_norm_m, grps, prot.info=hs_prot.info,
                  pr_ppos=3, my.pi=0.05, compute_pi=FALSE)
mylabs = c( 'CG','CG','CG', 'mCG','mCG','mCG')
prot_to_plot = 'Prot32' # 43
gene_to_plot = 'Gene32'
plot_3_pep_trends_N0file(as.matrix(hs_m_ints_eig1$m),
                        hs_m_ints_eig1$prot.info,
                        as.matrix(hs_norm_m),
                        hs_prot.info,
                        imp_hs$y_imputed,
                        imp_hs$imp_prot.info,
                        prot_to_plot, 3,
                        gene_to_plot, 4, mylabs)

```

plot_volcano

Volcano plot

Description

Function plots fold changes and p-values as a volcano plot. Two lines are plotted for the p-value cutoff at $p = PV_cutoff$ (solid line) and $p = 0.1$ (dashed line).

Usage

```
plot_volcano(FC, PV, FC_cutoff = 2, PV_cutoff = 0.05, figtitle = "")
```

Arguments

FC	vector of fold changes
PV	vector of p-values, same length as FC
FC_cutoff	fold change cutoff where to draw vertical cutoff lines, default = 2
PV_cutoff	p-value cutoff where to draw a horizontal cutoff line, default == 0.05
figtitle	title to display at the top of the figure, default = ""

Value

Nil

Examples

```
data(mm_peptides)
head(mm_peptides)
intsCols = 8:13 # different from parameter names as
                # R uses outer name spaces if variable is undefined
metaCols = 1:7
m_logInts = make_intencities(mm_peptides, intsCols)
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)

# Normalize data
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))

set.seed(123)
mm_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected

# Impute missing values
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)
mm_prot.info = mm_m_ints_norm$normalized[,1:7]
mm_norm_m = mm_m_ints_norm$normalized[,8:13]

set.seed(125) # needed for reproducibility of imputation
imp_mm = MBimpute(mm_norm_m, grps, prot.info=mm_prot.info,
                  pr_ppos=2, my.pi=0.05, compute_pi=FALSE)
DE_res = peptideLevel_DE(imp_mm$y_imputed, grps, imp_mm$imp_prot.info,
                        pr_ppos=2)
plot_volcano(DE_res$FC, DE_res$BH_P_val, FC_cutoff=1.5,
             PV_cutoff=.05, figtitle='Mouse DE')
```

plot_volcano_wLab

Volcano plot with labels for the differentially expressed proteins

Description

Function plots fold changes and p-values as a volcano plot. Two lines are plotted for the p-value cutoff at $p = PV_cutoff$ (solid line) and $p = 0.1$ (dashed line).

Usage

```
plot_volcano_wLab(FC, PV, ProtID, FC_cutoff = 2, PV_cutoff = 0.05,
  figtitle = "")
```

Arguments

FC	vector of fold changes
PV	vector of p-values, same lenght as FC
ProtID	vector of protein IDs, can be gene IDs, same lenght as FC & PV. Nmaes in this vector will be displayed in the volcano plot for differentially expressed proteins for this reason short names are preferred.
FC_cutoff	fold change cutoff where to draw vertical cutoff lines, default = 2
PV_cutoff	p-value cutoff where to draw a horisontal cutoff line, default ==.05
figtitle	title to display at the top of the figure, default = "

Arguments

<code>mm_list</code>	list of matrices of intensities for each experiment, dimentions: numpeptides x numsamples
<code>treat</code>	list of data frames with treatment information to compute the statistic, parallel to <code>mm_list</code> and <code>prot.info</code>
<code>prot.info</code>	list of protein metadata for each matrix in <code>mm_list</code> , data.frame parallel to <code>mm_list</code> and <code>treat</code>
<code>prot_col_name</code>	column names present in all datasets that identifies protein IDs across all datasets
<code>nperm</code>	number of permutations
<code>dataset_suffix</code>	a list of strings that will be appended to the column names for FC, PV, BHPV and numebers of peptides

Value

a data frame with the following columns:

protIDused protein metadata, peptide sequence if was passed in as one of the columns is the first peptide equence encountered in the data for that protein

FCs Avegares across all datasets of the approximation of the fold change computed as percent missing observations group 1 munis in percent missing observations group 2 in `peptideLevel_PresAbsDE()` function

P_val p-value for the comparison between 2 groups (2 groups only here) obtained from a permutation test

BH_P_val Benjamini-Hochberg adjusted p-values

statistic statistic returned by the g-test and summed across all datasets, not very useful as depends on the direction of the test and can produce all 0's

u_prot_info column containing ptoein identifiers across all datasets

FCs Approximation of the fold change computed as percent missing observations group 1 munis in percent missing observations group 2 in `peptideLevel_PresAbsDE()` function

PV p-values produced by g-test for individual datasets

BHPV adjusted p-values produced by g-test for individual datasets

NUMPEP number of peptides observed for each protein in each of the datasets

Examples

```
# Load mouse dataset
data(mm_peptides)
head(mm_peptides)
intsCols = 8:13
metaCols = 1:7
m_logInts = make_intencities(mm_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG', 'CG', 'CG', 'mCG', 'mCG', 'mCG'))

set.seed(135)
mm_m_ints_eig1 = eig_norm1(m=m_logInts, treatment=grps, prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)
```

```

# Load human dataset
data(hs_peptides)
head(hs_peptides)
intsCols = 8:13
metaCols = 1:7
m_logInts = make_intencities(hs_peptides, intsCols)
m_prot.info = make_meta(hs_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))

set.seed(137)
hs_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
hs_m_ints_eig1$h.c # check the number of bias trends detected
hs_m_ints_norm = eig_norm2(rv=hs_m_ints_eig1)

# Set up for presence/absence analysis
raw_list = list()
norm_imp_prot.info_list = list()
raw_list[[1]] = mm_m_ints_eig1$m
raw_list[[2]] = hs_m_ints_eig1$m
norm_imp_prot.info_list[[1]] = mm_m_ints_eig1$prot.info
norm_imp_prot.info_list[[2]] = hs_m_ints_eig1$prot.info

protnames_norm_list = list()
protnames_norm_list[[1]] = unique(mm_m_ints_norm$normalized$MatchedID)
protnames_norm_list[[2]] = unique(hs_m_ints_norm$normalized$MatchedID)

presAbs_dd = get_presAbs_prots(mm_list=raw_list,
                              prot.info=norm_imp_prot.info_list,
                              protnames_norm=protnames_norm_list,
                              prot_col_name=2)

ints_presAbs = list()
protmeta_presAbs = list()
ints_presAbs[[1]] = presAbs_dd[[1]][[1]] # Mouse
ints_presAbs[[2]] = presAbs_dd[[1]][[2]] # HS
protmeta_presAbs[[1]] = presAbs_dd[[2]][[1]]
protmeta_presAbs[[2]] = presAbs_dd[[2]][[2]]

treats = list()
treats[[1]] = grps
treats[[2]] = grps

subset_presAbs = subset_proteins(mm_list=ints_presAbs,
                                prot.info=protmeta_presAbs, 'MatchedID')

nperm = 50 # set to 500+ for publication
set.seed(275937)
presAbs_comb = prot_level_multiMat_PresAbs(
  mm_list=subset_presAbs$sub_mm_list,
  treat=treats,
  prot.info=subset_presAbs$sub_prot.info,
  prot_col_name='MatchedID', nperm=nperm,
  dataset_suffix=c('MM', 'HS') )

plot_volcano(presAbs_comb$FC, presAbs_comb$BH_P_val,
             FC_cutoff=.5, PV_cutoff=.05,

```

```
'Combined Pres/Abs CG vs mCG')
```

 prot_level_multi_part *Multi-Matrix Differential Expression Analysis*

Description

Multi-Matrix Differential Expression Analysis computes Model-Based statistics for each dataset, the sum of individual statistics is the final statistic. The significance is determined via a permutation test which computed the same statistics and sums them after permuting the values across treatment groups. As is outlined in Karpievitch et al. 2018. Important to set the random number generator seed for reproducibility with `set.seed()` function.

Usage

```
prot_level_multi_part(mm_list, treat, prot.info, prot_col_name,
  nperm = 500, dataset_suffix)
```

Arguments

<code>mm_list</code>	list of matrices for each experiment, length = number of datasets to compare internal dataset dimensions: numpeptides x numsamples for each dataset
<code>treat</code>	list of data frames with treatment information to compute the statistic in same order as <code>mm_list</code>
<code>prot.info</code>	list of protein and peptide mapping for each matrix in <code>mm_list</code> , in same order as <code>mm_list</code>
<code>prot_col_name</code>	column name in <code>prot.info</code> that contains protein identifiers that link all datasets together. Not that Protein IDs will differ across different organisms and cannot be used as the linking identifier. Function <code>match_linker_ids()</code> produces numeric identifiers that link all datasets together
<code>nperm</code>	number of permutations, default = 500, this will take a while, test code with fewer permutations
<code>dataset_suffix</code>	vector of character strings that corresponds to the dataset being analysed. Same length as <code>mm_list</code> . Names will be appended to the columns names that will be generated for each analysed dataset. For example, if analysing mouse and human data this vector may be: <code>c('Mouse', 'Human')</code>

Value

data frame with the following columns

protIDused Column containing the protein IDs used to link proteins across datasets

FC Average fold change across all datasets

P_val Permutation-based p-value for the differences between the groups

BH_P_val Multiple testing adjusted p-values

statistic Statistic computed as a sum of statistics produced for each dataset

Protein Information all columns passed into the function for the 1st dataset in the list

FCs Fold changes for individual datasets, these values should average to the FC above. As many columns as there are datasets being analyzed.

PV p-values for individual datasets. As many columns as there are datasets being analyzed.

BHPV Multiple testing adjusted p-values for individual datasets. As many columns as there are datasets being analyzed.

NUMPEP Number of peptides presents in each protien for each dataset. As many columns as there are datasets being analyzed.

Examples

```
# Load mouse dataset
data(mm_peptides)
head(mm_peptides)
intsCols = 8:13 # different from parameter names as R uses
                # outer name spaces if variable is undefined
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(mm_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))
set.seed(135)
mm_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,
                          prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)
mm_prot.info = mm_m_ints_norm$normalized[,1:7]
mm_norm_m = mm_m_ints_norm$normalized[,8:13]
set.seed(125) # Needed for reproducibility of results
imp_mm = MBimpute(mm_norm_m, grps, prot.info=mm_prot.info,
                  pr_ppos=2, my.pi=0.05, compute_pi=FALSE)

# Load human dataset
data(hs_peptides)
head(hs_peptides)
intsCols = 8:13 # different from parameter names as R uses
                # outer name spaces if variable is undefined
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(hs_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(hs_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))
set.seed(1237) # needed for reproducibility
hs_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
hs_m_ints_eig1$h.c # check the number of bias trends detected
hs_m_ints_norm = eig_norm2(rv=hs_m_ints_eig1)
hs_prot.info = hs_m_ints_norm$normalized[,1:7]
hs_norm_m = hs_m_ints_norm$normalized[,8:13]

set.seed(125) # or any value, ex: 12345
imp_hs = MBimpute(hs_norm_m, grps, prot.info=hs_prot.info,
                  pr_ppos=2, my.pi=0.05,
                  compute_pi=FALSE)

# Multi-Matrix Model-based differential expression analysis
# Set up needed variables
mms = list()
```



```

treats = list()
protinfos = list()
mms[[1]] = imp_mm$y_imputed
mms[[2]] = imp_hs$y_imputed
treats[[1]] = grps
treats[[2]] = grps
protinfos[[1]] = imp_mm$imp_prot.info
protinfos[[2]] = imp_hs$imp_prot.info
nperm = 50

# ATTENTION: SET RANDOM NUMBER GENERATOR SEED FOR REPRODUCIBILITY !!
set.seed(131) # needed for reproducibility

comb_MBDE = prot_level_multi_part(mm_list=mms, treat=treats,
                                  prot.info=protinfos,
                                  prot_col_name='ProtID', nperm=nperm,
                                  dataset_suffix=c('MM', 'HS'))

# Analysis for proteins only present in mouse,
# there are no proteins suitable for
# Model-Based analysis in human dataset
subset_data = subset_proteins(mm_list=mms, prot.info=protinfos, 'MatchedID')
mm_dd_only = subset_data$sub_unique_mm_list[[1]]
hs_dd_only = subset_data$sub_unique_mm_list[[2]]
protinfos_mm_dd = subset_data$sub_unique_prot.info[[1]]
DE_mCG_CG_mm_dd = peptideLevel_DE(mm_dd_only, grps,
                                    prot.info=protinfos_mm_dd, pr_ppos=2)

```

subset_proteins	<i>Subset proteins</i>
-----------------	------------------------

Description

Subset proteins into ones common to all datasets passed into the function and unique to each dataset. Note: for 3+ datasets no intermediate combinations of proteins are returned, only proteins common to all datasets, the rest are returned as unique to each dataset.

Usage

```
subset_proteins(mm_list, prot.info, prot_col_name)
```

Arguments

mm_list	list of matrices for each experiment, length = number of datasets to compare internal dataset dimensions: numpeptides x numsamples for each dataset
prot.info	list of protein and peptide mapping for each matrix in mm_list, in same order as mm_list
prot_col_name	column name in prot.info that contains protein identifiers that link all datasets together. Not that Protein IDs will differ across different organisms and cannot be used as the linking identifier. Function match_linker_ids() produces numeric identifiers that link all datasets together

Value

data frame with the following columns

sub_mm_list list of dataframes of intensities for each of the datasets passed in with proteins present in all datasets

sub_prot.info list of dataframes of metadata for each of the datasets passed in with proteins present in all datasets. Same order as sub_mm_list

sub_unique_mm_list list of dataframes of intensities not found in all datasets

sub_unique_prot.info list of dataframes of metadata not found in all datasets

common_list list of protein IDs common to all datasets

Examples

```
# Load mouse dataset
data(mm_peptides)
head(mm_peptides)
# different from parameter names as R uses
# outer name spaces if variable is undefined
intsCols = 8:13
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(mm_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(mm_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))
set.seed(173)
mm_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
mm_m_ints_eig1$h.c # check the number of bias trends detected
mm_m_ints_norm = eig_norm2(rv=mm_m_ints_eig1)
mm_prot.info = mm_m_ints_norm$normalized[,1:7]
mm_norm_m = mm_m_ints_norm$normalized[,8:13]
set.seed(131)
imp_mm = MBimpute(mm_norm_m, grps,
                  prot.info=mm_prot.info, pr_ppos=2, my.pi=0.05,
                  compute_pi=FALSE)

# Load human dataset
data(hs_peptides)
head(hs_peptides)
intsCols = 8:13
metaCols = 1:7 # reusing this variable
m_logInts = make_intencities(hs_peptides, intsCols) # will reuse the name
m_prot.info = make_meta(hs_peptides, metaCols)
m_logInts = convert_log2(m_logInts)
grps = as.factor(c('CG','CG','CG', 'mCG','mCG','mCG'))
hs_m_ints_eig1 = eig_norm1(m=m_logInts,treatment=grps,prot.info=m_prot.info)
hs_m_ints_eig1$h.c # check the number of bias trends detected
hs_m_ints_norm = eig_norm2(rv=hs_m_ints_eig1)
hs_prot.info = hs_m_ints_norm$normalized[,1:7]
hs_norm_m = hs_m_ints_norm$normalized[,8:13]
set.seed(131)
imp_hs = MBimpute(hs_norm_m, grps,
                  prot.info=hs_prot.info, pr_ppos=2,
                  my.pi=0.05,
                  compute_pi=FALSE)
```

```

# Multi-Matrix Model-based differential expression analysis
# Set up needed variables
mms = list()
treats = list()
protinfos = list()
mms[[1]] = imp_mm$y_imputed
mms[[2]] = imp_hs$y_imputed
treats[[1]] = grps
treats[[2]] = grps
protinfos[[1]] = imp_mm$imp_prot.info
protinfos[[2]] = imp_hs$imp_prot.info

subset_data = subset_proteins(mm_list=mms, prot.info=protinfos, 'MatchedID')
mms_mm_dd = subset_data$sub_unique_mm_list[[1]]
protinfos_mm_dd = subset_data$sub_unique_prot.info[[1]]
# Differential expression analysis for mouse specific protiens
DE_mCG_CG_mm_dd = peptideLevel_DE(mms_mm_dd, grps,
                                   prot.info=protinfos_mm_dd, pr_ppos=2)

```

sva.id

Surrogate Variable Analysis

Description

Surrogate Variable Analysis function used internatly by eig_norm1 and eig_norm2 Here we incorporate the model matrix from EigenMS normalization to find the significant trends in the matrix of residuals.

Usage

```
sva.id(dat, n.u.treatment, lm.fm, B = 500, sv.sig = 0.05)
```

Arguments

dat	number of peptides/genes x number of samples matrix of expression data with no missing values
n.u.treatment	number of treatment groups
lm.fm	formular for treatment to be use on the right side of the call to stats::lm() as generated by makeLMFormula()
B	The number of null iterations to perform
sv.sig	The significance cutoff for the surrogate variables

Value

A data structure with the following values:

n.sv Number of significant surrogate variables

p.sv Significance for the returned surrogate variables

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