

# Package ‘ProteoMM’

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

**Version** 1.31.0

**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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**License** MIT

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convert\_log2

*Convert values in a matrix to log2 transformed values*

---

## 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 indices 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>
----------	---

---

**Description**

Compute PI - proportion of observations missing completely at random

**Usage**

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

**Arguments**

m	matrix of abundances, numsamples 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 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 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 `ctest'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>	<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+ colum 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+ colum 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 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 indices that contain intensities
m_logInts = make_intencities(hs_peptides, intsCols)
# replace 0's with NA's as NA's are more appropriate
# for analysis 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_NOfile(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 length as FC
ProtID	vector of protein IDs, can be gene IDs, same length as FC & PV. Names 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 horizontal cutoff line, default = 0.05
figtitle	title to display at the top of the figure, default = ""



**Arguments**

<code>mm_list</code>	list of matrices of intensities for each experiment, dimensions: 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 numbers 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 minus 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 minus 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 internally 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**

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