Package ‘crossmeta’

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Title Cross Platform Meta-Analysis of Microarray Data
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Description Implements cross-platform and cross-species meta-analyses of Affymetrix, Illumina, and Agilent microarray data. This package automates common tasks such as downloading, normalizing, and annotating raw GEO data. The user then selects control and treatment samples in order to perform differential expression analyses for all comparisons. After analysing each contrast seperately, the user can select tissue sources for each contrast and specify any tissue sources that should be grouped for the subsequent meta-analyses.

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addContrastInput

Description

Add contrast input

Usage

addContrastInput(id)
add_adjusted  
Add expression data adjusted for pairs/surrogate variables

Description
Add expression data adjusted for pairs/surrogate variables

Usage
add_adjusted(eset, svobj = list(sv = NULL), numsv = 0)

Arguments
- eset: ExpressionSet
- svobj: surrogate variable object
- numsv: Number of surrogate variables to adjust for

Value
eset with adjusted element added

add_sources  
Add sample source information for meta-analysis.

Description
User selects a tissue source for each contrast and indicates any sources that should be paired. This step is required if you would like to perform source-specific effect-size/pathway meta-analyses.

Usage
add_sources(diff_exprs, data_dir = getwd(), postfix = NULL)

Arguments
- diff_exprs: Previous result of diff_expr, which can be reloaded using load_diff.
- data_dir: String specifying directory of GSE folders.
- postfix: Optional string to append to saved results. Useful if need to run multiple meta-analyses on the same series but with different contrasts.
**add_vsd**

**Add VST normalized assay data element to expression set**

**Description**

For microarray datasets duplicates exprs slot into vsd slot.

**Usage**

```r
add_vsd(eset, rna_seq = TRUE)
```

**Arguments**

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>eset</td>
<td>ExpressionSet with group column in pData(eset)</td>
</tr>
<tr>
<td>rna_seq</td>
<td>Is this an RNA-seq eset? Default is TRUE.</td>
</tr>
</tbody>
</table>

**Details**

The **Sources** tab is used to add a source for each contrast. To do so: click the relevant contrast rows, search for a source in the *Sample source* dropdown box, and then click the *Add* button.

The **Pairs** tab is used to indicate sources that should be paired (treated as the same source for subsequent effect-size and pathway meta-analyses). To do so: select at least two sources from the *Paired sources* dropdown box, and then click the *Add* button.

For each GSE, analysis results with added sources/pairs are saved in the corresponding GSE folder (in data_dir) that was created by `get_raw`.

**Value**

Same as `diff_expr` with added slots for each GSE in `diff_exprs`:

- **sources**: Named vector specifying selected sample source for each contrast. Vector names identify the contrast.
- **pairs**: List of character vectors indicating tissue sources that should be treated as the same source for subsequent effect-size and pathway meta-analyses.

**Examples**

```r
library(lydata)

# load result of previous call to diff_expr:
data_dir <- system.file("extdata", package = "lydata")
gse_names <- c("GSE9601", "GSE34817")
anals <- load_diff(gse_names, data_dir)

# run shiny GUI to add tissue sources
# anals <- add_sources(anals, data_dir)
```

```r
add_vsd(eset, rna_seq = TRUE)
```
Value

eset with 'vsd' assayDataElement added.

bulkAnnot

Logic for downloading and uploading bulk annotation

Description

Logic for downloading and uploading bulk annotation

Usage

bulkAnnot(input, output, session, dataset_name, pdata)

bulkAnnotInput

UI for Bulk Data annotation upload/download

Description

UI for Bulk Data annotation upload/download

Usage

bulkAnnotInput(id)

bulkForm

Logic for Bulk Data form

Description

Logic for Bulk Data form

Usage

bulkForm(input, output, session, pdata, prev)
bulkFormInput

**Description**
Input form for Bulk Data page

**Usage**
bulkFormInput(id)

---

bulkPage

**Description**
Logic for Select Contrasts Interface

**Usage**
bulkPage(input, output, session, eset, gse_name, prev)

**Arguments**
- input, output, session: shiny module boilerplate
- eset: ExpressionSet
- gse_name: GEO accession for the series.
- prev: Previous result of `diff_expr` used to allow rechecking previous selections.

---

bulkPageUI

**Description**
UI for Select Contrasts Interface

**Usage**
bulkPageUI(id)

**Arguments**
- id: The id string to be namespaced.
bulkTable  Logic for pdata table

Description
Logic for pdata table

Usage
bulkTable(input, output, session, eset, prev, up_annot)

bulkTableOuput  Tables for datasets page

Description
Tables for datasets page

Usage
bulkTableOuput(id)

ch2_subset  Subset for Paired Two-Channel ExpressionSet

Description
Two-channel esets use intraspotCorrelation and lmscFit so can’t use duplicateCorrelation. If not using one channel in contrasts (e.g. because all reference RNA) and have paired design, better to treat as single channel so that can use duplicateCorrelation.

Usage
ch2_subset(eset, prev_anal)

Arguments
eset    Annotated ExpressionSet. Created by load_raw.
prev_anal    One item (for eset) from previous result of diff_expr.

Value
ExpressionSet. If two-channel, paired and one channel not used will subset to used channel.
**clean_y**  
*Adjusts expression data for surrogate variables.*

**Description**
Factors out effect of surrogate variables discovered during surrogate variable analysis.

**Usage**

```
clean_y(y, mod, mod.clean)
```

**Arguments**

- **y**  
  Expression data of eset.
- **mod**  
  Full model matrix supplied to sva.
- **mod.clean**  
  Model matrix with factors to clean.

**Value**
Expression data with effects of sv's removed.

---

**delContrastsInput**  
*Delete contrasts input*

**Description**
Delete contrasts input

**Usage**

```
delContrastsInput(id)
```
Differential expression analysis of esets.

Description

After selecting control and test samples for each contrast, surrogate variable analysis (sva) and differential expression analysis is performed.

Usage

diff_expr(
esets,
data_dir = getwd(),
annot = "SYMBOL",
prev_anals = list(NULL),
svanal = TRUE,
recheck = FALSE,
postfix = NULL,
port = 3838
)

Arguments

esets List of annotated esets. Created by load_raw.
data_dir String specifying directory of GSE folders.
annot String, column name in fData common to all esets. For duplicated values in this column, the row with the highest interquartile range across selected samples will be kept. If meta-analysis will follow, appropriate values are "SYMBOL" (default - for gene level analysis) or, if all esets are from the same platform, "PROBE" (for probe level analysis).
prev_anals Previous result of diff_expr, which can be reloaded using load_diff. If present, previous selections, names, and pairs will be reused.
svanal Use surrogate variable analysis? Default is TRUE.
recheck Would you like to recheck previous group/contrast annotations? Requires prev_anals. Default is FALSE.
postfix Optional string to append to saved results. Useful if need to run multiple meta-analyses on the same series but with different contrasts.
port See runApp().

Details

Click the Download icon and fill in the Group name column and optionally the Pairs column. Then save and upload the filled in metadata csv. After doing so, select a test and control group to compare and click the + icon to add the contrast. Repeat previous step to add additional contrasts.
After control and test samples have been added for all contrasts that you wish to include, click the
Done button. Repeat for all GSEs.

Paired samples (e.g. the same subject before and after treatment) can be specified by filling out the
Pairs column before uploading the metadata.

For each GSE, analysis results are saved in the corresponding GSE folder in data_dir that was cre-
ated by get_raw. If analyses needs to be repeated, previous results can be reloaded with load_diff
and supplied to the prev_anals parameter. In this case, previous selections, names, and pairs will
be reused.

Value
List of named lists, one for each GSE. Each named list contains:

- pdata: data.frame with phenotype data for selected samples. Columns treatment ('ctrl'
or 'test'), group, and pair are added based on user selections.

- top_tables: List with results of topTable call (one per contrast). These results account for
the effects of nuissance variables discovered by surrogate variable analysis.

- ebayes_sv: Results of call to eBayes with surrogate variables included in the model matrix.

- annot: Value of annot variable.

Examples

library(lydata)

# location of raw data
data_dir <- system.file("extdata", package = "lydata")

# gather GSE names
gse_names <- c("GSE9601", "GSE15069", "GSE50841", "GSE34817", "GSE29689")

# load first eset
esets <- load_raw(gse_names[1], data_dir)

# run analysis (opens GUI)
# anals_old <- diff_expr(esets, data_dir)

# re-run analysis on first eset
prev <- load_diff(gse_names[1], data_dir)
anals <- diff_expr(esets[1], data_dir, prev_anals = prev)

es_meta

Effect size combination meta analysis.

Description
Performs effect-size meta-analyses across all studies and seperately for each tissue source.
Usage

```
es_meta(diff_exprs, cutoff = 0.3, by_source = FALSE)
```

Arguments

- **diff_exprs**: Previous result of `diff_expr`, which can be reloaded using `load_diff`.
- **cutoff**: Minimum fraction of contrasts that must have measured each gene. Between 0 and 1.
- **by_source**: Should separate meta-analyses be performed for each tissue source added with `add_sources`?

Details

Builds on `zScores` function from GeneMeta by allowing for genes that were not measured in all studies. This implementation also uses moderated unbiased effect sizes calculated by `effectsize` from metaMA and determines false discovery rates using `fdrtool`.

Value

A list of named lists, one for each tissue source. Each list contains two named data.frames. The first, `filt`, has all the columns below for genes present in cutoff or more fraction of contrasts. The second, `raw`, has only `dprime` and `vardprime` columns, but for all genes (NAs for genes not measured by a given contrast).

- **dprime**: Unbiased effect sizes (one column per contrast).
- **vardprime**: Variances of unbiased effect sizes (one column per contrast).
- **mu**: Overall mean effect sizes.
- **var**: Variances of overall mean effect sizes.
- **z**: Overall z score = \( \frac{\mu}{\sqrt{\text{var}}} \).
- **fdr**: False discovery rates calculated from column `z` using `fdrtool`.
- **pval**: p-values calculated from column `z` using `fdrtool`.

Examples

```
library(lydata)

# location of data
data_dir <- system.file("extdata", package = "lydata")

# gather GSE names
gse_names <- c("GSE9601", "GSE15069", "GSE50841", "GSE34817", "GSE29689")

# load previous analysis
anals <- load_diff(gse_names, data_dir)

# add tissue sources to perform separate meta-analyses for each source (optional)
# anals <- add_sources(anals, data_dir)
```
# perform meta-analysis
es <- es_meta(anals, by_source = TRUE)

---

**exprs.MA**

*Extract Log-Expression Matrix from MAList*

**Description**

Converts M and A-values to log-expression values. The output matrix will have two columns for each array, in the order all red then all green. Adapted from `plotDensities.MAList` instead of `exprs.MA` so that order is same as `phenoData.ch2`.

**Usage**

`exprs.MA(MA)`

**Arguments**

- **MA** an `MAList` object.

**Value**

A numeric matrix with twice the columns of the input.

---

**filter_genes**

*Filter genes in RNA-seq ExpressionSet*

**Description**

Uses `filterByExpr` to filter based on `counts` assay or `exprs` assay if `counts` isn’t available (for ARCHS4 data).

**Usage**

`filter_genes(eset)`

**Arguments**

- **eset** ExpressionSet with `counts` assayDataElement and group column in pData

**Value**

`filtered eset`
See Also

filterByExpr

Examples

# example ExpressionSet
eset <- makeExampleCountsEset()
eset <- filter_genes(eset)

Description

Fit ebayes model

Usage

fit_ebayes(
  lm_fit, 
  contrasts, 
  robust = TRUE, 
  trend = FALSE, 
  allow.no.resid = FALSE
)

Arguments

lm_fit Result of call to run_limma
contrasts Character vector of contrasts to fit.
robust logical, should the estimation of df.prior and var.prior be robustified against outlier sample variances?
trend logical, should an intensity-dependent trend be allowed for the prior variance? If FALSE then the prior variance is constant. Alternatively, trend can be a row-wise numeric vector, which will be used as the covariate for the prior variance.
allow.no.resid Allow no residual degrees of freedom? if TRUE and the fit contrast matrix has no residual degrees of freedom, eBayes fit is skipped and the result of contrasts.fit is returned.

Value

result of eBayes
**fit_lm**

*Run limma analysis.*

**Description**

Runs limma differential expression analysis on all contrasts selected by add_contrast. Analysis performed with and without surrogate variables discovered by diff_setup. Also prints MDS plot and saves results.

**Usage**

```r
fit_lm(eset, svobj = list(sv = NULL), numsv = 0, rna_seq = TRUE)
```

**Arguments**

- `eset`  Annotated eset created by load_raw. Replicate features and non-selected samples removed by iqr_replicates.
- `svobj` Surrogate variable analysis results. Returned from run_sva.
- `numsv` Number of surrogate variables to model.
- `rna_seq` Is this an RNA-seq eset? Default is TRUE.

**Value**

- list with slots: * fit Result of lmFit. * mod model matrix used for fit.

**fix_illum_headers**

*Attempts to fix Illumina raw data header*

**Description**

Reads raw data files and tries to fix them up so that they can be loaded by read.ilmn.

**Usage**

```r
fix_illum_headers(elist_paths, eset = NULL)
```

**Arguments**

- `elist_paths` Path to Illumina raw data files. Usually contain patterns: non_normalized.txt, raw.txt, or _supplementary_.txt
- `eset` ExpressionSet from getGEO.

**Value**

- Character vector for annotation argument to read.ilmn. Fixed raw data files are saved with file-name ending in _fixed.txt
format_dl_annot  

*Format downloaded annotation*

**Description**
Format downloaded annotation

**Usage**

```r
format_dl_annot(annot)
```

format_up_annot  

*Format uploaded annotation*

**Description**
Format uploaded annotation

**Usage**

```r
format_up_annot(up, ref)
```

get_ch2_mod  

*Get design matrix for two-channel array*

**Description**
Get design matrix for two-channel array

**Usage**

```r
get_ch2_mod(eset)
```

**Arguments**

- **eset** ExpressionSet with colnames that end in ‘_red’ and '_green’ indicating channel and eset$group indicating group membership.

**Value**

- model matrix for use by `intraspotCorrelation` and `lmscFit`
### get_group_levels

*Get group levels for bulk data plots*

**Description**

Get group levels for bulk data plots

**Usage**

```r
get_group_levels(pdata)
```

**Arguments**

- **pdata**
  
  Data.frame of phenotype data

---

### get_palette

*Get a Pallete to Distinguish Groups*

**Description**

Get a Pallete to Distinguish Groups

**Usage**

```r
get_palette(levs, dark = FALSE, with_all = FALSE)
```

**Arguments**

- **levs**
  
  Character vector of levels to get colour pallete for.

**Value**

Character vector with colour codes of `length(levs)`. 
get_raw

Download and unpack microarray supplementary files from GEO.

Description

Downloads and unpacks microarray supplementary files from GEO. Files are stored in the supplied data directory under the GSE name.

Usage

get_raw(gse_names, data_dir = getwd())

Arguments

gse_names  Character vector of GSE names to download.
data_dir   String specifying directory for GSE folders.

Value

NULL (for download/unpack only).

See Also

load_raw.

Examples

get_raw("GSE41845")

get_svaMods

Get model matrices for surrogate variable analysis

Description

Used by add_adjusted to create model matrix with surrogate variables.

Usage

get_svaMods(pdata)

Arguments

pdata       data.frame of phenotype data with column 'group' and 'pair' (optional).

Value

List with model matrix(mod) and null model matrix (mod0) used for sva.
get_top_table

get_top_table

Description
Get top table

Usage

get_top_table(
  lm_fit,
  groups = c("test", "ctrl"),
  with.es = TRUE,
  robust = FALSE,
  trend = FALSE,
  allow.no.resid = FALSE
)

Arguments

lm_fit Result of run_limma

groups Test and Control group as strings.

with.es Add 'dprime' and 'vardprime' from effectsize? Default is TRUE.

robust logical, should the estimation of df.prior and var.prior be robustified against outlier sample variances?

trend logical, should an intensity-dependent trend be allowed for the prior variance? If FALSE then the prior variance is constant. Alternatively, trend can be a row-wise numeric vector, which will be used as the covariate for the prior variance.

allow.no.resid Allow no residual degrees of freedom? if TRUE and the fit contrast matrix has no residual degrees of freedom, eBayes fit is skipped and the result of contrasts.fit is returned.

Value
result of toptable
**get_vsd**

*Get variance stabilized data for exploratory data analysis*

**Description**

Get variance stabilized data for exploratory data analysis

**Usage**

```r
get_vsd(eset)
```

**Arguments**

- `eset` ExpressionSet loaded with `load_raw`

**Value**

Matrix with variance stabilized expression data.

---

**gs.names**

*Map between KEGG pathway numbers and names.*

**Description**

Used to map human KEGG pathway numbers to names. Updated Feb 2017.

**Usage**

```r
data(gs.names)
```

**Format**

An object of class character of length 310.

**Value**

A named character vector of human KEGG pathway names. Names of vector are KEGG pathway numbers.
gslist


Usage
data(gslist)

Format
An object of class list of length 310.

Value
A named list with entrez ids of genes for human KEGG pathways. List names are KEGG pathway numbers.

ilmn.nnum

Count numeric columns in raw Illumina data files

Description
Excludes probe ID cols

Usage
ilmn.nnum(elist_paths)

Arguments
elist_paths Paths to raw illumina data files

Value
Number of numeric columns in elist_paths excluding probe ID columns.
iqr_replicates  

*Removes features with replicated annotation.*

**Description**

For rows with duplicated annot, highested IQR retained.

**Usage**

```r
iqr_replicates(eset, annot = "SYMBOL", rm.dup = FALSE)
```

**Arguments**

- **eset**: Annotated eset created by `load_raw`.
- **annot**: feature to use to remove replicates.
- **rm.dup**: remove duplicates (same measure, multiple ids)? Used for Pathway analysis so that doesn’t treat probes that map to multiple genes as distinct measures.

**Value**

Expression set with unique features at probe or gene level.

---

is_invertible  

*Check uploaded bulk pdata to make sure the study design is invertible*

**Description**

Check uploaded bulk pdata to make sure the study design is invertible

**Usage**

```r
is_invertible(pdata)
```
load_agil_plat  Load Agilent raw data

Description
Load Agilent raw data

Usage
load_agil_plat(eset, gse_name, gse_dir, ensql)

Arguments
- eset  ExpressionSet from getGEO.
- gse_name  Accession name for eset.
- gse_dir  Direction with Agilent raw data.
- ensql  For development. Path to sqlite file with ENTREZID and SYMBOL columns created in data-raw/entrezdt.

Value
ExpressionSet

load_diff  Load previous differential expression analyses.

Description
Loads previous differential expression analyses.

Usage
load_diff(gse_names, data_dir = getwd(), annot = "SYMBOL", postfix = NULL)

Arguments
- gse_names  Character vector specifying GSE names to be loaded.
- data_dir  String specifying directory of GSE folders.
- annot  Level of previous analysis (e.g. "SYMBOL" or "PROBE").
- postfix  Optional string to append to saved results. Useful if need to run multiple meta-analyses on the same series but with different contrasts.

Value
Result of previous call to diff_expr.
Examples

```r
library(lydata)

data_dir <- system.file("extdata", package = "lydata")
gse_names <- c("GSE9601", "GSE34817")
prev <- load_diff(gse_names, data_dir)
```

load_illum_plat

Illumina loader utility for load_plat.

Description

Used by load_plat to load an eset.

Usage

```r
load_illum_plat(eset, gse_name, gse_dir, ensql)
```

Arguments

- `eset`: Expression set obtained by `getGEO`.
- `gse_name`: String specifying GSE name.
- `gse_dir`: String specifying path to GSE folder.

Value

Annotated eset.

See Also

- `load_plat`.

load_plat

Load and pre-process raw Affymetrix, Illumina, and Agilent microarrays.

Description

Load raw files previously downloaded with `get_raw`. Used by `load_raw`.

Usage

```r
load_plat(gse_name, data_dir, gpl_dir, ensql)
```
load_raw

Arguments

- **gse_name**: GSE names.
- **data_dir**: String specifying directory with GSE folder.
- **gpl_dir**: String specifying parent directory to search for previously downloaded GPL.soft files.
- **ensql**: For development. Path to sqlite file with ENTREZID and SYMBOL columns created in data-raw/entrezdt.

Details

Data is normalized, SYMBOL and PROBE annotation are added to fData slot.

Value

List of annotated esets, one for each platform in gse_name.

See Also

- `get_raw` to obtain raw data.

load_raw

Load and annotate raw data downloaded from GEO.

Description

Loads and annotates raw data previously downloaded with `get_raw`. Supported platforms include Affymetrix, Agilent, and Illumina.

Usage

```r
load_raw(
  gse_names,
  data_dir = getwd(),
  gpl_dir = "..",
  overwrite = FALSE,
  ensql = NULL
)
```

Arguments

- **gse_names**: Character vector of GSE names.
- **data_dir**: String specifying directory with GSE folders.
- **gpl_dir**: String specifying parent directory to search for previously downloaded GPL.soft files.
- **overwrite**: Do you want to overwrite saved esets from previous load_raw?
- **ensql**: For development. Path to sqlite file with ENTREZID and SYMBOL columns created in data-raw/entrezdt.
makeExampleCountsEset

Value

List of annotated esets.

Examples

library(lydata)
data_dir <- system.file("extdata", package = "lydata")
eset <- load_raw("GSE9601", data_dir = data_dir)

makeExampleCountsEset  Make example ExpressionSet

Description

adapted from DESeq2::makeExampleDESeqDataSet

Usage

makeExampleCountsEset(
  n = 1000,
  m = 12,
  betaSD = 0,
  interceptMean = 4,
  interceptSD = 2,
  dispMeanRel = function(x) 4/x + 0.1,
  sizeFactors = rep(1, m)
)

Arguments

n number of rows
m number of columns
betaSD the standard deviation for non-intercept betas, i.e. beta ~ N(0,betaSD)
interceptMean the mean of the intercept betas (log2 scale)
interceptSD the standard deviation of the intercept betas (log2 scale)
dispMeanRel a function specifying the relationship of the dispersions on 2^{trueIntercept}
sizeFactors multiplicative factors for each sample

Examples

eset <- makeExampleCountsEset()
**match_prev_eset**

Reuse contrast selections from previous analysis.

**Description**

Transfers user-supplied selections from previous call of `diff_expr`.

**Usage**

```r
match_prev_eset(eset, prev_anal)
```

**Arguments**

- `eset` Annotated eset. Created by `load_raw`.
- `prev_anal` One item (for `eset`) from previous result of `diff_expr`. If present, previous selections and names will be reused.

**Value**

Expression set with samples and pData as in `prev_anal`.

**See Also**

`diff_expr`

**open_raw_illum**

Open raw Illumina microarray files.

**Description**

Helper function to open raw Illumina microarray files in order to check that they are formatted correctly. For details on correct format, please see 'Checking Raw Illumina Data' in vignette.

**Usage**

```r
open_raw_illum(gse_names, data_dir = getwd())
```

**Arguments**

- `gse_names` Character vector of Illumina GSE names to open.
- `data_dir` String specifying directory with GSE folders.

**Value**

Character vector of successfully formatted Illumina GSE names.
library(lydata)

# Illumina GSE names
illum_names <- c("GSE50841", "GSE34817", "GSE29689")

# location of raw data
data_dir <- system.file("extdata", package = "lydata")

# open raw data files with default text editor
# open_raw_illum(illum_names)

---

**phenoData.ch2**

*Construct AnnotatedDataFrame from Two-Channel ExpressionSet*

**Description**

Construct AnnotatedDataFrame from Two-Channel ExpressionSet

**Usage**

phenoData.ch2(eset)

**Arguments**

- **eset** ExpressionSet with pData for two-channel Agilent array.

**Value**

AnnotatedDataFrame with twice as many rows as eset, one for each channel of each array in order all red then all green.

---

**prefix_illum_headers**

*Run prefix on Illumina raw data files*

**Description**

Run prefix on Illumina raw data files

**Usage**

prefix_illum_headers(elist_paths)

**Arguments**

- **elist_paths** Paths to raw Illumina data files
**Value**

Paths to fixed versions of `elist_paths`

---

**query_ref**

Get correlation between query and reference signatures.

---

**Description**

Determines the pearson correlation between the query and each reference signature.

**Usage**

`query_ref(query, ref, sorted = TRUE, ngenes = 200)`

**Arguments**

- **query**
  
  Named numeric vector of differential expression values for query genes. Usually 'meta' slot of `get_dprimes` result.

- **ref**
  
  A matrix of differential expression to query against (rows are genes, columns are samples).

- **sorted**
  
  Would you like the results sorted by decreasing similarity? Default is TRUE.

- **ngenes**
  
  The number of top differentially-regulated (up and down) query genes to use.

**Value**

Vector of pearson correlations between query and reference signatures.

---

**remove_autonamed**

Remove columns that are autonamed by data.table

---

**Description**

Auto-named columns start with 'V' followed by the column number.

**Usage**

`remove_autonamed(ex)`

**Arguments**

- **ex**
  
  data.frame loaded with `fread`

**Value**

ex with auto-named columns removed.
run_limma  

Description

After selecting control and test samples for a contrast, surrogate variable analysis (sva) and linear model fitting with lmFit is performed.

Usage

run_limma(
  eset,
  annot = "SYMBOL",
  svobj = list(sv = NULL),
  numsv = 0,
  filter = TRUE
)

Arguments

eset  Annotated eset created by load_raw.
annot String, column name in fData. For duplicated values in this column, the row with the highest interquartile range across selected samples will be kept. Appropriate values are "SYMBOL" (default - for gene level analysis) or "ENTREZID_HS" (for probe level analysis).
svobj Surrogate variable analysis results. Returned from run_sva.
numsv Number of surrogate variables to model.
filter For RNA-seq. Should genes with low counts be filtered? dseqr shiny app performs this step separately. Should be TRUE (default) if used outside of dseqr shiny app.

Details

If analyses need to be repeated, previous results can be reloaded with readRDS and supplied to the prev_anal parameter. In this case, previous selections will be reused.

Value

List with:

- fit result of lmFit.
- mod model.matrix used for fit
**run_limma_setup**

Setup ExpressionSet for running limma analysis

**Usage**

```r
run_limma_setup(eset, prev)
```

**Arguments**

- `eset` : ExpressionSet
- `prev` : previous result of call to `diff_expr`

**Value**

`eset` ready for `run_limma`

---

**run_lmfit**

Perform lmFit analysis from limma.

**Description**

If paired samples, runs `duplicateCorrelation` to estimate intra-patient variance.

**Usage**

```r
run_lmfit(eset, mod, rna_seq = TRUE)
```

**Arguments**

- `eset` : Annotated eset created by `load_raw`. Non-selected samples and duplicate features removed by `add_contrasts` and `iqr_replicates`.
- `mod` : Model matrix generated by `diff_setup`. With or without surrogate variables.
- `rna_seq` : Is this an RNA-seq eset? Default is `TRUE`.

**Value**

result from call to limma `lmFit`.
**run_select_contrasts**  
*Shiny gadget to upload groups and select contrasts*

**Description**
Shiny gadget to upload groups and select contrasts

**Usage**
```r
run_select_contrasts(
  eset,
  gse_name,
  prev = NULL,
  app_dir = system.file("select_contrasts", package = "crossmeta", mustWork = TRUE),
  port = 3838
)
```

**Arguments**
- **eset**: ExpressionSet
- **gse_name**: GEO accession for the series.
- **prev**: Previous result of `diff_expr`. Used to allow rechecking previous selections.
- **app_dir**: Directory to shiny app. For local development use 'inst/select_contrasts'. Default is in 'select_contrasts' sub directory of crossmeta package.
- **port**: See `runApp()`.

**Value**
result of `setup_prev`. Used to specify sample groups and contrasts for differential expression analysis.

---

**run_sva**  
*Run surrogate variable analysis*

**Description**
Run surrogate variable analysis

**Usage**
```r
run_sva(mods, eset, svanal = TRUE)
```
Arguments

mods  
result of `get_svaMods`

eset  
ExpressionSet

svanal  
Should surrogate variable analysis be run? If FALSE, returns dummy result.

Description

Function is useful when number of samples makes manual selection with `diff_expr` error prone and time-consuming. This is often true for large clinical data sets.

Usage

```r
setup_prev(eset, contrasts)
```

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>eset</td>
<td>List containing one expression set with pData 'group' and 'pair' (optional) columns. Name of eset should be the GSE name.</td>
</tr>
<tr>
<td>contrasts</td>
<td>Character vector specifying contrasts to analyse. Each contrast must take the form &quot;B-A&quot; where both &quot;B&quot; and &quot;A&quot; are present in eset pData 'group' column. &quot;B&quot; is the treatment group and &quot;A&quot; is the control group.</td>
</tr>
</tbody>
</table>

Value

List containing necessary information for prev_anal parameter of `diff_expr`.

Examples

```r
library(lydata)
library(Biobase)

# location of raw data
data_dir <- system.file("extdata", package = "lydata")

# load eset
gse_name <- c("GSE34817")
eset <- load_raw(gse_name, data_dir)

# inspect pData of eset
# View(pData(eset$GSE34817))  # if using RStudio
head(pData(eset$GSE34817))  # otherwise

# get group info from pData (differs based on eset)
group <- pData(eset$GSE34817)$characteristics_ch1.1
```
# make group names concise and valid
group <- gsub("treatment: ", "", group)
group <- make.names(group)

# add group to eset pData
pData(eset$GSE34817)$group <- group

# setup selections
sel <- setup_prev(eset, contrasts = "LY-DMSO")

# run differential expression analysis
anal <- diff_expr(eset, data_dir, prev_anal = sel)

symbol_annot

Add hgnc symbol to expression set.

Description
Function first maps entrez gene ids to homologous human entrez gene ids and then to hgnc symbols.

Usage
symbol_annot(eset, gse_name = "", ensql = NULL)

Arguments
eset Expression set to annotate.
gse_name GSE name for eset.
ensql For development. Path to sqlite file with ENTREZID and SYMBOL columns created in data-raw/entrezdt.

Details
Initial entrez gene ids are obtained from bioconductor annotation data packages or from feature data of supplied expression set. Homologous human entrez ids are obtained from homologene and then mapped to hgnc symbols using org.Hs.eg.db. Expression set is expanded if 1:many mappings occur.

Value
Expression set with hgnc symbols ("SYMBOL") and row names ("PROBE") added to fData slot.

See Also
load_raw.
Examples

```r
library(lydata)

# location of raw data
data_dir <- system.file("extdata", package = "lydata")

# load eset
eset <- load_raw("GSE9601", data_dir)[[1]]

# annotate eset (need if load_raw failed to annotate)
eset <- symbol_annot(eset)
```

---

**to_eset**

*Convert limma object to ExpressionSet*

**Description**

Convert limma object to ExpressionSet

**Usage**

```r
to_eset(object, eset)
```

**Arguments**

- `object`: an EList of MAList object containing expression data.
- `eset`: ExpressionSet from getGEO. Used for annotation.

**Value**

ExpressionSet using expression data from object and annotation from eset.

---

**to_ma**

*Convert expression values to MAList*

**Description**

Convert expression values to MAList

**Usage**

```r
to_ma(y)
```

**Arguments**

- `y`: Expression values from two-channel agilent array in order all red then all green.
which_max_iqr

Value

MAList

Examples

A <- matrix(rnorm(100), ncol = 5)
M <- matrix(rnorm(100), ncol = 5)
MA <- new('MAList', list(M=M, A=A))
colnames(MA) <- letters[1:5]

y <- exprs.MA(MA)
MA2 <- crossmeta:::to_ma(y)
all.equal(MA, MA2)

validate_up_annot

Validate uploaded bulk annotation

Description

Validate uploaded bulk annotation

Usage

validate_up_annot(up, ref)

which_max_iqr

Get row indices of maximum IQR within annotation groups

Description

Groups by group_by and determines row with maximum IQR.

Usage

which_max_iqr(eset, group_by, x = exprs(eset))

Arguments

eset ExpressionSet
group_by Column in fData(eset) to group by
x matrix of expression values to use for IQR

Value

Integer vector of row numbers representing rows with the maximum IQR after grouping by group_by
xls_to_txt

Covert .xls files to .txt

---

**Description**

For converting Illumina _Supplementary_* .xls files to .txt for load_illum_plat.

**Usage**

```r
taxs_to_txt(xls_paths)
```

**Arguments**

- `xls_paths` : Paths to .xls files
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