Package ‘crossmeta’

May 16, 2024

Title Cross Platform Meta-Analysis of Microarray Data
Version 1.30.0
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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 separately, the user can select tissue sources for each contrast and specify any tissue sources that should be grouped for the subsequent meta-analyses.

Depends R (>= 4.0)
SystemRequirements libxml2; libxml2-dev (deb), libxml2-devel (rpm)
libcurl: libcurl4-openssl-dev (deb), libcurl-devel (rpm)
openssl: libssl-dev (deb), openssl-devel (rpm), libssl_dev (csw), openssl@1.1 (brew)
License MIT + file LICENSE
Encoding UTF-8
LazyData TRUE
RoxygenNote 7.1.2
VignetteBuilder knitr

URL https://github.com/alexvpickering/crossmeta
BugReports https://github.com/alexvpickering/crossmeta/issues
Suggests knitr, rmarkdown, lydata, org.Hs.eg.db, testthat
Imports affy (>= 1.52.0), affxparser (>= 1.46.0), AnnotationDbi (>= 1.36.2), Biobase (>= 2.34.0), BiocGenerics (>= 0.20.0), BiocManager (>= 1.30.4), DT (>= 0.2), DBI (>= 1.0.0), data.table (>= 1.10.4), edgeR, fdrtool (>= 1.2.15), GEOquery (>= 2.40.0), limma (>= 3.30.13), matrixStats (>= 0.51.0), metaMA (>= 3.1.2), miniUI (>= 0.1.1), methods, oligo (>= 1.38.0), reader(>= 1.0.6), RCurl (>= 1.95.4.11), RSQLite (
2.1.1), stringr (>= 1.2.0), sva (>= 3.22.0), shiny (>= 1.0.0),
shinyjs (>= 2.0.0), shinyBS (>= 0.61), shinyWidgets (>= 0.5.3),
shinypanel (>= 0.1.0), tibble, XML (>= 3.98.1.17), readxl (>=
1.3.1)

biocViews GeneExpression, Transcription, DifferentialExpression,
Microarray, TissueMicroarray, OneChannel, Annotation,
BatchEffect, Preprocessing, GUI

git_url https://git.bioconductor.org/packages/crossmeta

git_branch RELEASE_3_19

git_last_commit 7500c56

git_last_commit_date 2024-04-30

Repository Bioconductor 3.19

Date/Publication 2024-05-16

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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

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)
```

---

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

Description

For microarray datasets duplicates `exprs` slot into `vsd` slot.

Usage

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

Arguments

- **eset**: ExpressionSet with group column in `pData(eset)`
- **rna_seq**: Is this an RNA-seq `eset`? Default is 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**

**Logic for Select Contrasts Interface**

**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**

**UI for Select Contrasts Interface**

**Description**

UI for Select Contrasts Interface

**Usage**

bulkPageUI(id)

**Arguments**

- id: The id string to be namespaced.
null
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)
**diff_expr**

*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**

```r
 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 created 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)

# 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

\texttt{es\_meta(diff\_exprs, cutoff = 0.3, by\_source = FALSE)}

Arguments

\texttt{diff\_exprs} \hspace{1cm} \text{Previous result of \texttt{diff\_expr}, which can be reloaded using \texttt{load\_diff}.}

\texttt{cutoff} \hspace{1cm} \text{Minimum fraction of contrasts that must have measured each gene. Between 0 and 1.}

\texttt{by\_source} \hspace{1cm} \text{Should separate meta-analyses be performed for each tissue source added with \texttt{add\_sources}?}

Details

Builds on \texttt{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 \texttt{effectsize} from metaMA and determines false discovery rates using \texttt{fdrtool}.

Value

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

\texttt{dprime} \hspace{1cm} \text{Unbiased effect sizes (one column per contrast).}

\texttt{vardprime} \hspace{1cm} \text{Variances of unbiased effect sizes (one column per contrast).}

\texttt{mu} \hspace{1cm} \text{Overall mean effect sizes.}

\texttt{var} \hspace{1cm} \text{Variances of overall mean effect sizes.}

\texttt{z} \hspace{1cm} \text{Overall z score = } \frac{\mu}{\sqrt{\text{var}}}.

\texttt{fdr} \hspace{1cm} \text{False discovery rates calculated from column z using fdrtool.}

\texttt{pval} \hspace{1cm} \text{p-values calculated from column z using fdrtool.}

Examples

\texttt{library(lydata)}

# location of data
\texttt{data\_dir <- system.file("extdata", package = "lydata")}

# gather GSE names
\texttt{gse\_names <- c("GSE9601", "GSE15069", "GSE50841", "GSE34817", "GSE29689")}

# load previous analysis
\texttt{anals <- load\_diff(gse\_names, data\_dir)}

# add tissue sources to perform separate meta-analyses for each source (optional)
# \texttt{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)

fit_ebayes

Fit ebayes model

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**

A 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**

A character vector for annotation argument to `read.ilmn`. Fixed raw data files are saved with filename ending in _fixed.txt.
format_d1_annot  
*Format downloaded annotation*

**Description**
Format downloaded annotation

**Usage**
```
format_d1_annot(annot)
```

---

format_up_annot  
*Format uploaded annotation*

**Description**
Format uploaded annotation

**Usage**
```
format_up_annot(up, ref)
```

---

get_ch2_mod  
*Get design matrix for two-channel array*

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

**Usage**
```
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 `lmseFit`
get_group_levels

Get group levels for bulk data plots

Description

Get group levels for bulk data plots

Usage

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

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

```r
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

```r
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

```r
get_sva_mods(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.
Description

Get top table

Usage

```r
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

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

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

KEGG human pathway genes.

#### Description

#### Usage
```r
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
```r
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

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

is_invertible(pdata)
**load_agil_plat**  
*Load Agilent raw data*

**Description**  
Load Agilent raw data

**Usage**  
```r
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**  
```r
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

library(lydata)

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

Description

Used by load_plat to load an eset.

Usage

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

load_plat(gse_name, data_dir, gpl_dir, ensql)
**load_raw**

**Arguments**
- **gse_names**: 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_names.

**See Also**
- `get_raw` to obtain raw data.

---

**Description**
Load and annotate raw data downloaded from GEO.

**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.
Value

List of annotated esets.

Examples

```r
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

```r
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

```r
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 formated Illumina GSE names.
Examples

```r
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**

```r
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**

```r
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
```r
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
```r
remove_autonamed(ex)
```

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

### Value
ex with auto-named columns removed.
run_limma  

Linear model fitting of eset with 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

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

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  \textit{Shiny gadget to upload groups and select contrasts}

**Description**

Shiny gadget to upload groups and select contrasts

**Usage**

\begin{verbatim}
run_select_contrasts(
eset,
gse_name,
prev = NULL,
app_dir = system.file("select_contrasts", package = "crossmeta", mustWork = TRUE),
port = 3838
)
\end{verbatim}

**Arguments**

- **eset**  ExpressionSet
- **gse_name**  GEO accession for the series.
- **prev**  Previous result of \texttt{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 \texttt{runApp()}. 

**Value**

result of \texttt{setup_prev}. Used to specify sample groups and contrasts for differential expression analysis.

run_sva  \textit{Run surrogate variable analysis}

**Description**

Run surrogate variable analysis

**Usage**

\begin{verbatim}
run_sva(mods, eset, svanal = TRUE)
\end{verbatim}
**setup_prev**

**Arguments**

- mods: result of `get_sva.mods`
- 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**

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

**Arguments**

- eset: List containing one expression set with pData 'group' and 'pair' (optional) columns. Name of eset should be the GSE name.
- contrasts: Character vector specifying contrasts to analyse. Each contrast must take the form "B-A" where both "B" and "A" are present in eset pData 'group' column. "B" is the treatment group and "A" is the control group.

**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
```
symbol_annot

# 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
**Description**

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

**Usage**

```python
xls_to_txt(xls_paths)
```

**Arguments**

- `xls_paths` Paths to .xls files
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