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/pathway 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. Finally, effect size and pathway meta-analyses can proceed and the results graphically explored.

Depends R (>= 3.3)

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Encoding UTF-8

LazyData TRUE

RoxygenNote 6.0.1

VignetteBuilder knitr

Suggests knitr, rmarkdown, lydata, org.Hs.eg.db, testthat, ccdata

Imports affy (>= 1.52.0), affxparser (>= 1.46.0), AnnotationDbi (>= 1.36.2), Biobase (>= 2.34.0), BiocGenerics (>= 0.20.0), Bioconductor >= 1.42.0, CCmap, DT (>= 0.2), data.table (>= 1.10.4), doParallel (>= 1.0.10), doRNG (>= 1.6), dsstool (>= 1.2.15), foreach (>= 1.4.3), ggplot2 (>= 2.2.1), GEOquery (>= 2.40.0), limma (>= 3.30.13), matrixStats (>= 0.51.0), metaMA (>= 3.1.2), metap (>= 0.8), miniUI (>= 0.1.1), oligo (>= 1.38.0), pander (>= 0.6.0), plotly (>= 4.5.6), reshape (>= 0.8.6), RColorBrewer (>= 1.1.2), rdrop2 (>= 0.7.0), stringr (>= 1.2.0), sva (>= 3.22.0), shiny (>= 1.0.0), stats (>= 3.3.3)

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

NeedsCompilation no
add_sources

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())

Arguments

- `diff_exprs`: Previous result of `diff_expr`, which can be reloaded using `load_diff`.
- `data_dir`: String specifying directory of GSE folders.

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`.

R topics documented:

- add_sources
- contribute
- diff_expr
- diff_path
- es_meta
- explore_paths
- get_raw
- gs.names
- gslist
- load_diff
- load_path
- load_raw
- open_raw_illum
- path_meta
- setup_prev
- symbol_annot

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Values

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)
```

```
contribute

Contribute results of meta-analysis to public database.

Description

Contributed results will be used to build a freely searchable database of gene expression meta-analyses.

Usage

`contribute(diff_exprs, subject)`

Arguments

- `diff_exprs`: Result of call to `diff_expr`.
- `subject`: String identifying meta-analysis subject (e.g. "rapamycin" or "prostate_cancer").

Details

Performs meta-analysis on `diff_exprs` using `es_meta`. Sends overall mean effect size values and minimal information needed to reproduce meta-analysis.

Value

NULL (used to contribute meta-analysis).
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 differential expression analyses
anals <- load_diff(gse_names, data_dir)

# contribute results of meta-analysis
# contribute(anals, subject = "LY294002")

### 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))
```

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.

Details

The **Samples** tab is used to select control and test samples for each contrast. To do so: select rows for control samples, type a group name in the **Control group name** text input box and click the **Add Group** button. Repeat for test samples. While adding additional contrasts, a previous control group can be quickly reselected from the **Previous selections** dropdown box. 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 selecting sample rows to pair and then clicking **Pair Samples**. The author does not usually specify paired...
samples and instead allows surrogate variable analysis to discover these inter-sample relationships from the data itself.

The **Contrasts** tab is used to view and delete contrasts that have already been added.

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 `pairs` are added based on user selections.
- **top_tables**
  - List with results of `topTable` call (one per contrast). These results account for the effects of nuisance 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

```r
library(lydata)

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

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

deets <- load_raw(gse_names[1], data_dir)

eets <- load_raw(gse_names[1], data_dir)

diff_path(esets, data_dir)

diff_path(esets[1], data_dir, prev_anals = prev)
```

---

**diff_path**

Differential expression of KEGG pathways.

### Description

Performs PADOG pathway analysis using KEGG database (downloaded Feb 2017).

### Usage

```r
diff_path(esets, prev_anals, data_dir = getwd())
```
Arguments

esets  List of annotated esets. Created by load_raw.
prev_anals  Previous result of diff_expr, which can be reloaded using load_diff.
data_dir  String specifying directory for GSE folders.

Details

If you wish to perform source-specific pathway meta-analyses, add_sources must be used before diff_path.

For each GSE, analysis results are saved in the corresponding GSE folder in data_dir that was created by get_raw. PADOG outperforms other pathway analysis algorithms at prioritizing expected pathways (see references).

Value

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

- padog_tables  data.frames containing padog pathway analysis results for each contrast.

If add_sources is used first:

- 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 pathway meta-analysis.

References


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 esets
esets <- load_raw(gse_names, data_dir)

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

# add tissue sources to perform separate meta-analyses for each source (recommended)
# anals <- add_sources(anals)

# perform pathway analysis for each contrast
# path_anals <- diff_path(esets, anals, data_dir)
**es_meta**

Effect size combination meta analysis.

**Description**

Performs effect-size meta-analyses across all studies and separately for each tissue source.

**Usage**

```r
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 = `mu` / `sqrt(var)`.
- `fdr`: False discovery rates calculated by `fdrtool`.

**Examples**

```r
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
```
explore_paths

## Description

Shiny app for interactively exploring the results of effect-size and pathway meta-analyses. The app also interfaces with the ccmap package in order to explore drugs that are predicted to reverse or mimic your signature.

## Usage

```r
explore_paths(es_res, path_res, drug_info = NULL, type = c("both", "mimic", "reverse"))
```

## Arguments

- `es_res`: Result of call to `es_meta`
- `path_res`: Result of call to `path_meta`
- `drug_info`: Matrix of differential expression values for drugs (rows are genes, columns are drugs). If NULL (default), `cmap_es` is used.
- `type`: Desired direction of drug action on query signature (see details).

## Details

For a given tissue source (top left dropdown box) and KEGG pathway (bottom left dropdown box, ordered by increasing false discovery rate), effect-sizes (y-axis) are plotted for each gene in the pathway (x-axis, ordered by decreasing absolute effect size).

For each gene, open circles give the effect-sizes for each contrast. The transparency of the open circles is proportional to the standard deviation of the effect-size for each contrast. For each gene, error bars give one standard deviation above and below the the overall meta-analysis effect-size.

The top drugs for the full signature in a given tissue (top right dropdown box, red points) and just the pathway genes (bottom right dropdown box, blue points) are ordered by decreasing (if `type` is 'both' or 'mimic') or increasing (if `type` is 'reverse') similarity. Positive and negative cosine similarities correspond to drugs that, respectively, mimic and reverse the query signature.

Drug effect sizes can be made visible by either clicking the legend entries (top left of plot) or selecting a new drug in the dropdown boxes.

When a new tissue source or pathway is selected, the top drug and pathway dropdown boxes are appropriately updated.

## Value

None
**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")
```
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 312.

**Value**

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

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gslist

*KEGG human pathway genes.*

**Description**


**Usage**

```r
data(gslist)
```

**Format**

An object of class `list` of length 312.

**Value**

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

Load previous differential expression analyses.

Description
Loads previous differential expression analyses.

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

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").

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_path

Load previous pathway analyses.

Description
Load previous pathway analyses.

Usage
load_path(gse_names, data_dir = getwd())

Arguments
- **gse_names**: Character vector of GSE names.
- **data_dir**: String specifying directory for GSE folders.

Value
Result of previous call to `diff_path`. 

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

load_raw(gse_names, data_dir = getwd(), gpl_dir = ".", overwrite = FALSE)

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?

Value

List of annotated esets.

Examples

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

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.

Examples

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)

path_meta  

Pathway p-value meta analysis.

Description

Uses Fisher's method to combine p-values from PADOG pathway analyses.

Usage

path_meta(path_anals, ncores = parallel::detectCores(), nperm = ncores * 10000, by_source = FALSE)
setup_prev

Arguments

- `path_anals` Previous result of `diff_path`, which can be reloaded using `load_path`.
- `ncores` Number of cores to use. Default is all available.
- `nperm` Number of permutation to perform to calculate p-values.
- `by_source` Should separate meta-analyses be performed for each tissue source added with `add_sources`?

Details

Permutation p-values are determined by shuffling pathway names associated with PADOG p-values prior to meta-analysis. Permutation p-values are then adjusted using the Benjamini & Hochberg method to obtain false discovery rates.

Value

A list of matrices, one for each tissue source. Each matrix contains a column of PADOG p-values for each contrast and permutation p- and fdr-values for the meta analysis.

See Also

`sumlog`, `padog`.

Examples

```
library(lydata)

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

data_dir <- system.file("extdata", package = "lydata")

gse_names <- c("GSE9601", "GSE15069", "GSE50841", "GSE34817", "GSE29689")

# load previous pathway analyses
# path_anals <- load_path(gse_names, data_dir)

# perform pathway meta analysis
# path_res <- path_meta(path_anals, ncores = 1, nperm = 100)
```

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)`
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 = "")
symbol_annot

Arguments

eset    Expression set to annotate.
gse_name  GSE name for eset.

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

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)
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