

Package: path2path (via r-universe)

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Type Package

Title Directional Pathway-by-Pathway Interaction Maps from
Genome-Scale Perturb-Seq

Version 0.99.4

Description Builds a directional pathway-by-pathway interaction map from genome-scale Perturb-seq differential-expression statistics by two stacked runs of preranked gene set enrichment analysis: once per perturbation over genes, then once per readout pathway over perturbations. Entry (j,k) of the resulting matrix measures whether perturbations targeting pathway j's members produce a coordinated transcriptional response in pathway k. The package includes target exclusion, effective-size filters, overlap masking, Stage 2 p-values with false-discovery-rate control, and a whole-pipeline permutation calibration with generalized-Pareto tail estimation.

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as_path2path_matrix	Convert a SummarizedExperiment assay to a path2path input matrix.
---------------------	---

Description

A SummarizedExperiment stores features in rows and samples or perturbations in columns. path2path uses the transpose: perturbations in rows and measured genes in columns. This helper performs that conversion while retaining the assay dimnames.

Usage

```
as_path2path_matrix(x, assay = 1L)
```

Arguments

x a ‘SummarizedExperiment’ with genes in rows and perturbations in columns.

assay character(1) or integer(1): assay name or index passed to ‘SummarizedExperiment::assay()’.

Value

a numeric perturbation-by-gene matrix.

Examples

```
if (requireNamespace("SummarizedExperiment", quietly = TRUE)) {
  data(demo_z)
  se <- SummarizedExperiment::SummarizedExperiment(
    assays = list(z = t(demo_z))
  )
  z <- as_path2path_matrix(se, "z")
  stopifnot(identical(z, demo_z))
}
```

as_path2path_se	<i>Convert a path2path map to a SummarizedExperiment.</i>
-----------------	---

Description

The returned object stores the map and its p-value matrices as assays, allowing the pathway-by-pathway result to be used with standard Bioconductor infrastructure. The Stage 1 matrix remains available in the original result object.

Usage

```
as_path2path_se(result)
```

Arguments

result a list, a standard ‘path2path()’ result containing ‘M’.

Value

a ‘SummarizedExperiment’ with pathways as rows and readout pathways as columns.

Examples

```
if (requireNamespace("SummarizedExperiment", quietly = TRUE)) {
  data(demo_z)
  data(demo_pathways)
  res <- path2path(
    demo_z, demo_pathways,
    stage1 = list(nperm = 100, min_size = 5),
    stage2 = list(min_pu = 5),
    BPPARAM = BiocParallel::SerialParam(), verbose = FALSE
  )
  se <- as_path2path_se(res)
  SummarizedExperiment::assayNames(se)
}
```

bh_populated	<i>Benjamini-Hochberg over the populated (non-NA) cells of a matrix.</i>
--------------	--

Description

Exported utility: encodes the package's multiple-testing family rule (one BH family over all populated entries), for use on any p-value matrix produced by the package (e.g. `negative-control stage2_matrix()` output).

Usage

```
bh_populated(P)
```

Arguments

P a numeric matrix of p-values (NA = unpopulated/masked entries).

Value

matrix of the same shape with BH-adjusted values on populated entries.

Examples

```
P <- matrix(c(0.001, 0.04, NA, 0.5), 2, 2)
bh_populated(P)
```

build_null_pool	<i>Whole-pipeline permutation null calibration.</i>
-----------------	---

Description

Runs the same path2path() pipeline on B permuted matrices and pools |M'| entries into a sorted null pool. Each null M' is saved to out_dir for reuse (per-pathway regrouping, tail fits).

Usage

```
build_null_pool(
  z,
  pathways,
  B = 10L,
  out_dir = NULL,
  config = NULL,
  seed_base = NULL,
  verbose = FALSE,
  ...
)
```

Arguments

z, pathways	the inputs of the observed run: 'z' a numeric matrix (perturbations x genes, rownames = perturbed gene symbols) and 'pathways' a named list of character vectors of gene symbols.
B	integer(1): number of outer permutations.
out_dir	'NULL' or character(1): optional directory persisting each null map (M_null_bNNN.rds), per-map BH discovery counts, and each null map's sorted nominal p-values; completed maps found there are reloaded instead of recomputed (resume). Use a separate directory for each input dataset, pathway collection, and pipeline configuration.
config	'NULL' or a list, a 'path2path()' result's '\$config' component, so null runs repeat the observed configuration exactly.
seed_base	'NULL' or integer(1): if non-NULL, uses 'seed_base + b' for the input permutation in run b. For exact reproducibility of the GSEA calculations, also use seeded 'BPPARAM' objects in 'config' or '...'.
verbose	logical(1): report per-permutation progress messages (default FALSE).
...	additional path2path() arguments; these override entries of 'config' with the same name.

Value

a list with two components: 'null_M', a length-'B' list of pathway x pathway numeric matrices (one null map per permutation), and 'pool_abs', a sorted numeric vector pooling the absolute values of all non-missing null-map entries.

Examples

```
data(demo_z)
data(demo_pathways)
cfg <- list(stage1 = list(nperm = 100, min_size = 5),
            stage2 = list(min_pu = 5),
            BPPARAM = BiocParallel::SerialParam())
pool <- build_null_pool(demo_z, demo_pathways, B = 2, seed_base = 1,
                       stage1 = cfg$stage1, stage2 = cfg$stage2,
                       BPPARAM = cfg$BPPARAM)
length(pool$pool_abs)
```

calibrate_pvalues	<i>One-call calibrated p-values for an existing map.</i>
-------------------	--

Description

Runs (or reuses) the outer-permutation calibration and returns the map's entries with pooled-empirical and GPD-calibrated p-values attached. Expensive: B full pipeline runs. 'pool' may be supplied from a previous build_null_pool() to skip recomputation.

Usage

```
calibrate_pvalues(
  result,
  z = NULL,
  pathways = NULL,
  B = 50L,
  pool = NULL,
  q_thresh = 0.99,
  null_dir = NULL,
  seed_base = NULL,
  verbose = FALSE
)
```

Arguments

result	a list as returned by path2path() (uses result\$M, and reuses result\$config so the null runs repeat the observed run's configuration exactly; there is deliberately no way to override it here, since mismatched null runs would invalidate the calibration. For full control, call build_null_pool() directly and pass its pool via 'pool').
z, pathways	the inputs that produced 'result': 'z' a numeric matrix (perturbations x genes) and 'pathways' a named list of character vectors of gene symbols. Both may be 'NULL' when a precomputed 'pool' is supplied.
B	integer(1): number of outer permutations (ignored if 'pool' given).
pool	'NULL' or numeric vector: optional sorted M' pool from build_null_pool()\$pool_abs.

q_thresh	numeric(1) in (0, 1): GPD threshold quantile of the pool (default 0.99); a fit-adequacy guard lowers it stepwise (0.975, 0.95, 0.90) if the fitted tail's finite endpoint falls below the largest observed IML.
null_dir	'NULL' or character(1), optional directory: each null map is saved there as M_null_bNNN.rds (persistence only; forwarded to build_null_pool's out_dir).
seed_base	'NULL' or integer(1): forwarded to build_null_pool().
verbose	logical(1): forwarded to build_null_pool() (per-permutation progress messages; default FALSE).

Value

list with K x K matrices 'pval_gpd' (the calibrated p-values: identical to 'pval_pool' up to the pool's 'q_thresh' quantile, GPD tail extrapolation beyond it when an adequate fit is available), 'padj_gpd' (single BH family over the populated entries of 'pval_gpd', the calibrated analogue of result\$padj), and 'pval_pool' (pooled empirical p-values, kept for diagnostics); plus 'gpd' (fit parameters) and 'pool_abs' (the pool, reusable via 'pool =').

References

Knijnenburg TA, Wessels LFA, Reinders MJT, Shmulevich I (2009). Fewer permutations, more accurate p-values. *Bioinformatics* 25:i161-i168. doi:10.1093/bioinformatics/btp211.

Examples

```
data(demo_z)
data(demo_pathways)
res <- path2path(demo_z, demo_pathways,
  stage1 = list(nperm = 100, min_size = 5),
  stage2 = list(min_pu = 5),
  BPPARAM = BiocParallel::SerialParam(), verbose = FALSE)
cal <- calibrate_pvalues(res, demo_z, demo_pathways, B = 2, seed_base = 1)
summary(as.vector(cal$pval_gpd))
```

dataset_tag	<i>Dataset tag from a zscore_<tag>.csv[.gz] filename.</i>
-------------	---

Description

Dataset tag from a zscore_<tag>.csv[.gz] filename.

Usage

```
dataset_tag(path)
```

Arguments

path character(1): a file path such as zscore_myexp.csv.gz.

Value

the tag string ("myexp").

Examples

```
dataset_tag("zscore_k562_gwps.csv.gz")
```

demo_pathways	<i>Synthetic demonstration pathway collection</i>
---------------	---

Description

Six disjoint 30-gene pathways over the demo_z gene vocabulary. Members of PATHWAY_A, PATHWAY_C, and PATHWAY_E are among the perturbed genes; see demo_z.

Usage

```
data(demo_pathways)
```

Format

named list of 6 character vectors.

demo_z	<i>Synthetic demonstration input matrix</i>
--------	---

Description

A small simulated Perturb-seq differential-expression matrix (120 perturbations x 400 genes) with two planted pathway-level relations: PATHWAY_A activates PATHWAY_B and PATHWAY_C suppresses PATHWAY_D (CRISPRi orientation), plus a strong on-target knockdown on every row's own gene. Generated by inst/scripts/make_demo_data.R (seeded).

Usage

```
data(demo_z)
```

Format

numeric matrix, 120 x 400; rownames = perturbed gene symbols, colnames = measured gene symbols.

jaccard_mask	<i>Mask off-diagonal entries whose pathway pair overlaps above ‘tau’.</i>
--------------	---

Description

High-overlap pairs (Reactome parent/child etc.) score mechanically — j’s members ride k’s signal because they ARE k’s members — which is gene-set structure, not causal regulation. Diagonal is never masked.

Usage

```
jaccard_mask(M, pathways, tau = 0.5, jaccard = NULL)
```

Arguments

M	K x K numeric matrix to mask (dimnames = pathway names).
pathways	named list of character vectors of gene symbols.
tau	numeric(1) in [0, 1]: overlap threshold; entries with Jaccard > tau become NA.
jaccard	‘NULL’ or numeric matrix: optional precomputed pathway_jaccard() matrix.

Value

M with high-overlap off-diagonal entries set to NA.

Examples

```
data(demo_pathways)
M <- matrix(1, length(demo_pathways), length(demo_pathways),
  dimnames = list(names(demo_pathways), names(demo_pathways)))
sum(is.na(jaccard_mask(M, demo_pathways, tau = 0.1)))
```

load_pathways	<i>Load an MSigDB collection as a named list of gene-symbol vectors.</i>
---------------	--

Description

Gene symbols are de-duplicated within each set. When ‘size_filter’ is TRUE the sets are restricted to ‘min_size <= |S| <= max_size’ measured on the *global* (genome) membership; the effective per-universe filters are applied later inside stage1_nes()/stage2_matrix(), which is where they matter statistically.

Usage

```
load_pathways(  
  collection,  
  min_size = 15L,  
  max_size = 500L,  
  species = "Homo sapiens"  
)
```

Arguments

- collection character(1), one of "hallmark", "reactome", "kegg", "kegg_medicus", "kegg_legacy", "pid", "wikipathways". Note that "kegg" resolves to KEGG_MEDICUS (the current KEGG subcollection in MSigDB); use "kegg_legacy" for the classic 186-set collection.
- min_size, max_size integer(1) each: global size window applied when 'size_filter' is active for the collection.
- species character(1): passed to msigdbr::msigdbr.

Value

named list of character vectors

References

Dolgalev I (2026). msigdbr: MSigDB Gene Sets for Multiple Organisms in a Tidy Data Format. R package.

Examples

```
hm <- load_pathways("hallmark")  
length(hm)
```

order_by_activity	<i>Pathway names ordered by number of significant entries (descending).</i>
-------------------	---

Description

'margin = "rows"' orders by outgoing (regulator) counts, "cols" by incoming (readout) counts. Returns ALL pathway names of that margin (zero-count ones last), so the result is a complete ordering usable as plot_map(order = ...).

Usage

```
order_by_activity(
  result,
  calibrated = NULL,
  padj_cutoff = 0.05,
  pval_cutoff = NULL,
  margin = c("rows", "cols"),
  include_diag = FALSE
)
```

Arguments

result	a list as returned by path2path().
calibrated	‘NULL’ or a list as returned by calibrate_pvalues() (switches the cutoffs to Route B values).
padj_cutoff, pval_cutoff	‘NULL’ or numeric(1) each: significance cutoffs; non-NULL cutoffs conjoin, NULL disables one (at least one must be enabled).
margin	character(1): order by outgoing ("rows") or incoming ("cols") significant counts.
include_diag	logical(1): count the self-interaction diagonal.

Value

character vector: a complete ordering of that margin’s names.

Examples

```
data(demo_z)
data(demo_pathways)
res <- path2path(demo_z, demo_pathways,
  stage1 = list(nperm = 100, min_size = 5),
  stage2 = list(min_pu = 5),
  BPPARAM = BiocParallel::SerialParam(), verbose = FALSE)
order_by_activity(res, padj_cutoff = 0.2)[1:3]
```

path2path	<i>Full two-stage GSEA pipeline: z matrix -> pathway x pathway matrix.</i>
-----------	---

Description

Orchestrates stage1_nes() -> stage2_matrix() -> jaccard_mask() and global BH adjustment over populated, unmasked cells. This is the single canonical code path: production runs, calibration permutations, and sensitivity analyses must all go through this function.

Usage

```
path2path(
  z,
  pathways,
  stage1 = list(),
  stage2 = list(),
  jaccard_tau = 0.5,
  BPPARAM = BiocParallel::bpparam(),
  verbose = FALSE
)
```

Arguments

<code>z</code>	numeric matrix, perturbations x genes (rownames = perturbed gene symbols, colnames = measured gene symbols).
<code>pathways</code>	named list of character vectors of gene symbols.
<code>stage1, stage2</code>	named lists of overrides forwarded to <code>stage1_nes()</code> and <code>stage2_matrix()</code> .
<code>jaccard_tau</code>	numeric(1) in [0, 1]: mask threshold; <code>NA_real_</code> disables masking.
<code>BPPARAM</code>	a <code>BiocParallel</code> parameter object forwarded to both stages (a <code>BPPARAM</code> entry inside <code>stage1/stage2</code> takes precedence for that stage).
<code>verbose</code>	logical(1): emit per-stage timing messages (default FALSE).

Value

list(nes, pval_stage1, M_raw, M, pval, padj, ..., config). ‘config’ records the stage1/stage2 overrides and `jaccard_tau` of this call, so that `calibrate_pvalues()` can rerun the identical configuration on permuted data without the user re-passing it.

Reproducibility

`BiocParallel` draws worker seeds from entropy unless the backend carries an explicit seed, so `set.seed()` alone does not make a run reproducible. For exact reproducibility pass a seeded backend, e.g. `BPPARAM = BiocParallel::MulticoreParam(RNGseed = 1)` (or `SerialParam(RNGseed = 1)`).

Examples

```
data(demo_z)
data(demo_pathways)
res <- path2path(demo_z, demo_pathways,
  stage1 = list(nperm = 100, min_size = 5),
  stage2 = list(min_pu = 5),
  BPPARAM = BiocParallel::SerialParam(), verbose = FALSE)
res$M[1:3, 1:3]
sum(res$padj < 0.05, na.rm = TRUE)
```

pathway_jaccard	<i>Pairwise Jaccard similarity of pathway gene sets (dense crossproduct).</i>
-----------------	---

Description

Pairwise Jaccard similarity of pathway gene sets (dense crossproduct).

Usage

```
pathway_jaccard(pathways)
```

Arguments

pathways named list of character vectors of gene symbols.

Value

K x K similarity matrix (diagonal set to 0; it is never masked).

Examples

```
data(demo_pathways)
J <- pathway_jaccard(demo_pathways)
max(J)
```

permute_input	<i>Whole-matrix permutation: one global row and one global column permutation.</i>
---------------	--

Description

Preserves the marginal value distribution and within-row gene-gene correlation (the same column permutation is applied to every row) while breaking pathway membership, because the original labels stay attached to the permuted positions.

Usage

```
permute_input(z)
```

Arguments

z perturbations x genes numeric matrix.

Value

the permuted matrix (labels retained in place, so every label sits atop another row/column's data).

Examples

```
data(demo_z)
set.seed(1)
zp <- permute_input(demo_z)
identical(dimnames(zp), dimnames(demo_z))
```

plot_calibration	<i>Route B diagnostics: null-pool tail versus the fitted GPD.</i>
------------------	---

Description

Left: empirical survival curve of the null pool (log10 scale) with the fitted GPD tail overlaid beyond the threshold u . Right: QQ plot of the exceedances against fitted GPD quantiles. Both panels support the judgment the user makes before quoting a `pval_gpd`: does the tail fit?

Usage

```
plot_calibration(calibrated)
```

Arguments

`calibrated` a list as returned by `calibrate_pvalues()` carrying `pool_abs` and `gpd`.

Value

`invisible(NULL)`; draws two base-graphics panels.

Examples

```
pool <- sort(-log(seq(0.9999, 0.0001, length.out = 5000)))
M <- matrix(c(0.5, 1, 1.5, 2), 2,
  dimnames = list(c("A", "B"), c("A", "B")))
cal <- calibrate_pvalues(list(M = M), pool = pool)
plot_calibration(cal)
```

plot_entry	<i>GSEA-style enrichment plot for one map entry: why is $M[j, k]$ strong?</i>
------------	--

Description

Shows the Stage 2 ranking (perturbations ranked by their Stage 1 NES on readout pathway k) with tick marks at the perturbations targeting members of pathway j , via `fgsea::plotEnrichment()`. The title reports the entry's NES and its p-value (Route B if 'calibrated' is given).

Usage

```
plot_entry(result, pathways, j, k, calibrated = NULL)
```

Arguments

result	a list as returned by path2path().
pathways	named list of character vectors: the collection used to build ‘result’.
j, k	character(1) each, pathway names: the perturbed (row) and readout (column) side.
calibrated	‘NULL’ or a list as returned by calibrate_pvalues(); switches the title to Route B values.

Value

a ggplot object.

Examples

```
data(demo_z)
data(demo_pathways)
res <- path2path(demo_z, demo_pathways,
  stage1 = list(nperm = 100, min_size = 5),
  stage2 = list(min_pu = 5),
  BPPARAM = BiocParallel::SerialParam(), verbose = FALSE)
if (requireNamespace("ggplot2", quietly = TRUE))
  plot_entry(res, demo_pathways, names(demo_pathways)[1],
    names(demo_pathways)[2])
```

plot_map

Heatmap of a path2path map with significance dots.

Description

Processing pipeline (in this fixed priority): (1) ‘rows’/‘cols’ subset; (2) all-NA rows/columns dropped if ‘drop_empty_*’; (3) ordering – ‘order’ if given (a character vector, or a list(rows=, cols=) as returned invisibly by a previous plot_map call, so a second map can be drawn under the first one’s ordering), else two-directional hierarchical clustering if ‘cluster = TRUE’, else matrix order. Dots mark entries passing the significance cutoffs (see .sig_matrix semantics: non-NULL cutoffs conjoin; ‘calibrated’ switches to Route B p-values). Masked/filtered entries are gray.

Usage

```
plot_map(
  result,
  calibrated = NULL,
  padj_cutoff = 0.05,
  pval_cutoff = NULL,
  rows = NULL,
  cols = NULL,
  drop_empty_rows = TRUE,
  drop_empty_cols = TRUE,
```

```

    order = NULL,
    cluster = TRUE,
    labels = NULL,
    zlim = c(-3, 3),
    main = NULL,
    colorbar = TRUE,
    dot_cex = NULL
  )

```

Arguments

result	a list as returned by path2path().
calibrated	‘NULL’ or a list as returned by calibrate_pvalues() (switches the cutoffs to Route B values).
padj_cutoff, pval_cutoff	‘NULL’ or numeric(1) each: significance cutoffs; non-NULL cutoffs conjoin, NULL disables one (at least one must be enabled).
rows, cols	‘NULL’ or character vectors: optional name subsets applied before anything else.
drop_empty_rows, drop_empty_cols	logical(1) each: drop all-NA rows/columns.
order	‘NULL’, a character vector, or list(rows=, cols=) fixing the order.
cluster	logical(1): two-directional hierarchical clustering when no ‘order’.
labels	‘NULL’ or logical(1): force axis labels on/off (default: on up to 60 pathways).
zlim	numeric(2): color range in NES units.
main	‘NULL’ or character(1): plot title.
colorbar	logical(1): draw the NES colorbar.
dot_cex	‘NULL’ or numeric(1): significance-dot size (default scales with map size).

Value

(invisible) list(rows, cols): the plotted ordering, reusable as the ‘order’ argument for another map.

Examples

```

data(demo_z)
data(demo_pathways)
res <- path2path(demo_z, demo_pathways,
  stage1 = list(nperm = 100, min_size = 5),
  stage2 = list(min_pu = 5),
  BPPARAM = BiocParallel::SerialParam(), verbose = FALSE)
plot_map(res, padj_cutoff = 0.2)

```

read_input_matrix	<i>Read a z-score CSV (rows = perturbed gene symbols in first column).</i>
-------------------	--

Description

Read a z-score CSV (rows = perturbed gene symbols in first column).

Usage

```
read_input_matrix(path)
```

Arguments

path	character(1): CSV or CSV.GZ file; first column = perturbed gene symbols, remaining columns = measured genes. Reading gzipped files requires the R.utils package (listed in Suggests).
------	--

Value

numeric matrix with dimnames.

Examples

```
data(demo_z)
f <- tempfile(fileext = ".csv")
write.csv(data.frame(perturbation = rownames(demo_z), demo_z,
  check.names = FALSE), f, row.names = FALSE)
z <- read_input_matrix(f)
identical(dim(z), dim(demo_z))
```

select_active	<i>Pathways with at least 'min_sig' significant entries, as regulators and as readouts.</i>
---------------	---

Description

Counts significant entries per row (outgoing, pathway as regulator) and per column (incoming, pathway as readout) separately – in practice out-degrees and in-degrees differ by an order of magnitude, so a joint count would drown the readout side. The result plugs directly into plot_map(): 'plot_map(res, rows = sel\$rows, cols = sel\$cols)'.

Usage

```
select_active(
  result,
  calibrated = NULL,
  padj_cutoff = 0.05,
  pval_cutoff = NULL,
  min_sig = 1L,
  include_diag = FALSE
)
```

Arguments

result a list as returned by path2path().

calibrated 'NULL' or a list as returned by calibrate_pvalues() (switches the cutoffs to Route B values).

padj_cutoff, pval_cutoff 'NULL' or numeric(1) each: significance cutoffs; non-NULL cutoffs conjoin, NULL disables one (at least one must be enabled).

min_sig integer(1): minimum number of significant entries.

include_diag logical(1): count the self-interaction diagonal (default FALSE, matching the paper's off-diagonal focus; only applies to square maps).

Value

list(rows = character, cols = character)

Examples

```
data(demo_z)
data(demo_pathways)
res <- path2path(demo_z, demo_pathways,
  stage1 = list(nperm = 100, min_size = 5),
  stage2 = list(min_pu = 5),
  BPPARAM = BiocParallel::SerialParam(), verbose = FALSE)
select_active(res, padj_cutoff = 0.2)
```

stage1_nes

Stage 1: per-perturbation preranked GSEA.

Description

For each row *m* of 'z' (a perturbation), ranks the measured genes by the CRISPRi-negated statistic $s = -z[m,]$ and scores every pathway with preranked GSEA. Returns the *M* x *K* NES matrix (and p-values for method = "fgsea").

Usage

```
stage1_nes(
  z,
  pathways,
  method = c("fgsea", "pooled"),
  exclude_target = TRUE,
  negate = TRUE,
  nperm = 1000L,
  min_size = 15L,
  max_size = 500L,
  b_prime = 1L,
  BPPARAM = BiocParallel::bpparam()
)
```

Arguments

<code>z</code>	numeric matrix, perturbations x genes; rownames = perturbed gene symbols, colnames = measured gene symbols. NAs are dropped per row.
<code>pathways</code>	named list of character vectors of gene symbols.
<code>method</code>	character(1): "fgsea" (fgseaSimple, per-perturbation permutations) or "pooled" (one pooled null per pathway across perturbations; no p-values).
<code>exclude_target</code>	logical(1): drop the perturbed gene from its own ranked list before scoring. This removes the tautological self-hit: the target is at the top of L_m by construction, giving every pathway that contains it a free hit. Default TRUE.
<code>negate</code>	logical(1): apply the CRISPRi loss-of-function sign flip ($s = -z$). Default TRUE; set FALSE only for gain-of-function perturbations.
<code>nperm</code>	integer(1), fgsea method only: permutations per ranked list for <code>fgsea::fgseaSimple</code> . The default 1000 estimates the NES accurately; downstream inference does not consume Stage 1 p-values.
<code>min_size, max_size</code>	integer(1) each: effective gene-set size window, applied to the intersection with each ranked list.
<code>b_prime</code>	integer(1), pooled method only: random null draws per perturbation pooled into the per-pathway null (1 draw x thousands of perturbations is already a large pool).
<code>BPPARAM</code>	a BiocParallel parameter object controlling parallelisation over perturbations; default <code>BiocParallel::bpparam()</code> .

Details

When ranked statistics contain ties, the function emits one summary warning for the stage rather than one warning per perturbation.

Value

list(nes = M x K matrix, pval = M x K matrix or NULL)

References

Korotkevich G, Sukhov V, Sergushichev A (2019). Fast gene set enrichment analysis. bioRxiv. doi:10.1101/060012.

Examples

```
data(demo_z)
data(demo_pathways)
s1 <- stage1_nes(demo_z, demo_pathways, nperm = 100,
  min_size = 5, BPPARAM = BiocParallel::SerialParam())
dim(s1$nes)
```

stage2_matrix

Stage 2: per-pathway-column GSEA in the perturbation universe.

Description

For each column k of the Stage 1 NES matrix, ranks the perturbations by $NES(., k)$ and asks, for every pathway j , whether the perturbations of S_j members cluster at either end. Pathways are restricted to the perturbation universe (rownames of 'nes') and must retain at least 'min_pu' members.

Usage

```
stage2_matrix(
  nes,
  pathways,
  method = c("fgsea", "pooled"),
  score_type = c("std", "both"),
  min_pu = 10L,
  min_col_size = 2L,
  max_size = 500L,
  min_valid = 20L,
  eps = 0,
  b_prime = 1L,
  BPPARAM = BiocParallel::bpparam()
)
```

Arguments

nes	numeric matrix, $M \times K$ Stage 1 NES values (rownames = perturbed gene symbols, colnames = pathway names).
pathways	named list of character vectors: the full pathway collection (gene symbols); restricted internally.
method	character(1): "fgsea" (fgseaMultilevel, p-values) or "pooled".

score_type	character(1): "std" (default GSEA two-sided score), or "both" to run scoreType "pos" and "neg" separately and return both extremes (M_pos / M_neg), which resolves bidirectionally-regulated pairs that cancel under "std".
min_pu	integer(1): minimum members of a set in the perturbation universe (rownames of 'nes') for the set to be tested at all.
min_col_size	integer(1): minimum members of a set actually present in one column's non-NA ranking for that entry to be scored. The universe filter 'min_pu' is global; within a single column, NA entries can reduce a set's effective size below 'min_pu', and 'min_col_size' bounds it from below (default 2, ruling out degenerate single-member scores). Raise it (e.g. to 'min_pu') for a stricter per-entry guarantee.
max_size	integer(1): maximum effective set size within a column's ranking.
min_valid	integer(1): minimum non-NA entries a column needs to be ranked at all.
eps	numeric(1): fgsea::fgseaMultilevel accuracy floor (0 = exact estimation of arbitrarily small p-values).
b_prime	integer(1), pooled method only: random null draws per column pooled into the per-pathway-row null.
BPPARAM	a BiocParallel parameter object controlling parallelisation over readout columns; default BiocParallel::bpparam().

Details

When ranked statistics contain ties, the function emits one summary warning for the stage rather than one warning per readout column.

Value

list with K x K matrices: M, pval (std) or M_pos, M_neg, pval_pos, pval_neg (both). Rows cover all pathways; pathways failing the min_pu filter are all-NA rows.

Examples

```
data(demo_z)
data(demo_pathways)
s1 <- stage1_nes(demo_z, demo_pathways, nperm = 100, min_size = 5,
  BPPARAM = BiocParallel::SerialParam())
s2 <- stage2_matrix(s1$nes, demo_pathways, min_pu = 5,
  BPPARAM = BiocParallel::SerialParam())
s2$M[1:3, 1:3]
```

write_result

Write pipeline outputs with dataset/collection namespacing.

Description

Files: nes_matrix_<ns>.csv.gz, pval_matrix_stage1_<ns>.csv.gz, M_matrix_<ns>_raw.csv, M_matrix_<ns>.csv, pval/padj_matrix_<ns>.csv (or *_pos/_neg variants for score_type = "both").

Usage

```
write_result(result, dir, tag, collection, method = "fgsea", calibrated = NULL)
```

Arguments

result	a list as returned by path2path().
dir	character(1): output directory.
tag, collection, method	character(1) each: components of the file namespace.
calibrated	'NULL' or a list as returned by calibrate_pvalues().

Details

If 'calibrated' (a calibrate_pvalues() result) is supplied, Route B outputs are written alongside: pval_gpd_matrix_<ns>.csv, pval_pool_matrix_<ns>.csv, gpd_params_<ns>.csv, and the null pool as null_pool_<ns>.rds (for reuse via calibrate_pvalues(pool=)).

Value

(invisible) the namespace string used in filenames.

Examples

```
data(demo_z)
data(demo_pathways)
res <- path2path(demo_z, demo_pathways,
  stage1 = list(nperm = 100, min_size = 5),
  stage2 = list(min_pu = 5),
  BPPARAM = BiocParallel::SerialParam(), verbose = FALSE)
d <- tempdir()
write_result(res, d, "demo", "demo6", method = "fgsea")
list.files(d, pattern = "demo")
```

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