Package ‘OVESEG’

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Description An R package for multiple-group comparison to detect tissue/cell-specific marker genes among subtypes. It provides functions to compute OVESEG-test statistics, derive component weights in the mixture null distribution model and estimate p-values from weightedly aggregated permutations. Obtained posterior probabilities of component null hypotheses can also portrait all kinds of upregulation patterns among subtypes.
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OVESEG-package

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OVESEG-package  OVESEG: A package for marker gene test.

Description

Function OVESEGtest performs OVESEG-test for expression profiles from multiple groups to detect subtype-specific marker genes. While it may take a long time to execute permutations for p-value estimation, users can apply OVESEGtstat to obtain OVESEG-test statistics to rank genes and apply postProbNull to obtain each gene’s posterior probabilities of component null hypotheses. nullDistri estimates probabilities of any one group being upregulated under null hypotheses. patternDistri estimates probabilities of all kinds of upregulation patterns among groups.

References

RNAseq count data downsampled from GSE60424

Description
Three cell subtypes (B cells, CD4+ T cells, CD8+ T cells) were purified from 20 fresh blood samples. RNA was extracted from each of these cell subsets and processed into RNA sequencing libraries (Illumina TruSeq). Sequencing libraries were analyzed on an Illumina HiScan, with a target read depth of ~20M reads. Reads were demultiplexed, mapped to human gene models (ENSEMBL), and tabulated using HTSeq. We downsample the original data to 10000 genes. Subtype labels for purified populations are also included. (Data generation script can be found in ./data_raw folder.)

Usage
data(countBT)

Format
A list with one count mixture (count) and a categorical vector giving subtypes (group).

References

nullDistri Probability of one group being upregulated under null

Description
This function estimates probabilities of any one group being upregulated than other groups under null hypotheses.

Usage
nullDistri(ppnull)

Arguments
ppnull a list returned by postProbNull or OVESEGtest.

Details
The probability of one group being upregulated under null hypotheses is calculated by accumulating and normalizing genewise posterior probability of null hypotheses. The group with higher probability tends to get more False Positive MGs.
Value

A numeric vector indicating probabilities of each group being upregulated than others under null hypotheses.

Examples

data(RocheBT)
ppnull <- postProbNull(RocheBT$y, RocheBT$group, alpha='moderated')
pk <- nullDistri(ppnull)

Description

This function performs OVESEG-test to assess significance of molecular markers.

Usage

OVESEGtest(y, group, weights = NULL, alpha = "moderated",
            NumPerm = 999, seed = 111, detail.return = TRUE,
            BPPARAM = bpparam())

Arguments

y

A numeric matrix containing log-expression or logCPM (log2-counts per million) values. Data frame or SummarizedExperiment object will be internally coerced into a matrix. Rows correspond to probes and columns to samples. Missing values are not permitted.

group

A categorical vector or factor giving group membership of columns of y. At least two categories need to be presented.

weights

Optional numeric matrix containing prior weights for each spot.

alpha

Parameter specifying within-group variance estimator to be used. 'moderated': empirical Bayes moderated variance estimator as used in eBayes. Numeric value: a constant value added to pooled variance estimator (α + σ). NULL: no estimator; all variances are set to be 1.

NumPerm

An integer specifying the number of permutation resamplings (default 999).

seed

An integer seed for the random number generator.

detail.return

A logical indicating whether more details about posterior probability estimation will be returned.

BPPARAM

A BiocParallelParam object indicating whether parallelization should be used for permutation resamplings. The default is bpparam().
Details

OVESEG-test is a statistically-principled method that can detect tissue/cell-specific marker genes among many subtypes. OVESEG-test statistics are designed to mathematically match the definition of molecular markers, and a novel permutation scheme are employed to estimate the corresponding distribution under null hypotheses where the expression patterns of non-markers can be highly complex.

Value

a list containing the following components:

- `pv.overall`: a vector of p-values calculated by all permutations regardless of upregulated subtypes.
- `pv.oneside`: a vector of subtype-specific p-values calculated specifically for the upregulated subtype of each probe.
- `pv.oneside.max`: subtype-specific p-values when observed test statistic equal to zero.
- `pv.multiside`: `pv.oneside*K` (K-time comparison correction) and truncated at 1.
- `W`: a matrix of posterior probabilities for each component null hypothesis given an observed probe. Rows correspond to probes and columns to one hypothesis.
- `label`: a vector of group labels.
- `groupOrder`: a matrix with each row being group indexes ordered based on decreasing expression levels. Group indexes are positions in `label`.
- `F.p.value`: a matrix with each column giving p-values corresponding to F-statistics on certain groups.
- `lfdr`: a matrix with each column being local false discovery rates estimated based on one column of weighted F.p.value matrix.
- `fit`: a MArrayLM fitted model object produced by `lmFit`.

`F.p.value`, `lfdr` and `fit` are returned only when `detail.return` is TRUE.

Examples

data(RocheBT)
rttest <- OVESEGtest(RocheBT$y, RocheBT$group, NumPerm=99,
                    BPPARAM=BiocParallel::SerialParam())
## Not run:
#parallel computing
rttest <- OVESEGtest(RocheBT$y, RocheBT$group, NumPerm=99,
                    BPPARAM=BiocParallel::SnowParam())
## End(Not run)
OVESEGtstat  

**OVESEG-test statistics**

**Description**

This function computes OVESEG-test statistics.

**Usage**

```r
OVESEGtstat(y, group, weights = NULL, alpha = "moderated", order.return = FALSE, lmfit.return = FALSE)
```

**Arguments**

- `y`: a numeric matrix containing log-expression or logCPM (log2-counts per million) values. Data frame or SummarizedExperiment object will be internally coerced into a matrix. Rows correspond to probes and columns to samples. Missing values are not permitted.
- `group`: categorical vector or factor giving group membership of columns of `y`. At least two categories need to be presented.
- `weights`: optional numeric matrix containing prior weights for each spot.
- `alpha`: parameter specifying within-group variance estimator to be used. 'moderated': empirical Bayes moderated variance estimator as used in `eBayes`. Numeric value: a constant value added to pooled variance estimator (\(\alpha + \sigma\)). NULL: no estimator; all variances are set to be 1.
- `order.return`: a logical indicating whether the order of groups will be returned. If FALSE, only the highest expressed group index is return for each probe.
- `lmfit.return`: a logical indicating whether a `MArrayLM` fitted model object produced by `lmFit` should be returned.

**Details**

OVESEG-test statistics are designed to mathematically match the definition of molecular markers:

\[
\max_{k=1,\ldots,K} \left\{ \min_{l \neq k} \frac{\mu_k(j) - \mu_l(j)}{\sigma(j) \sqrt{\frac{1}{N_k} + \frac{1}{N_l}}} \right\}
\]

where \(j\) is probe index, \(K\) is the number of groups, and \(\mu_k\) is the mean expression of group \(k\).

**Value**

- `label`: a vector of group labels.
OVEtstatPermTopM

- **groupOrder**: If `order.return` is TRUE, a matrix with each row being group indexes ordered based on decreasing expression levels. If `order.return` is FALSE, a vector with each element being a probe’s highest expressed group index. Group indexes are positions in `label`.

- **fit**: A `MArrayLM` fitted model object produced by `lmFit`. Returned only when `lmfit.return` is TRUE.

**Examples**

```r
data(RocheBT)
rtstat <- OVESEGtstat(RocheBT$y, RocheBT$group, alpha='moderated')
rtstat <- OVESEGtstat(RocheBT$y, RocheBT$group, alpha=0.1)
```

**OVEtstatPermTopM**: OVESEG-test statistics after permuting top M groups

**Description**

This function permutes group labels among highest expressed M groups and then computes new OVESEG-test statistics.

**Usage**

```
OVEtstatPermTopM(y, group, groupOrder, M, weights = NULL, 
alpha = "moderated", NumPerm = 999, seed = 111, 
BPPARAM = bpparam())
```

**Arguments**

- **y**: a numeric matrix containing log-expression or logCPM (log2-counts per million) values. Data frame or SummarizedExperiment object will be internally coerced into a matrix. Rows correspond to probes and columns to samples. Missing values are not permitted.

- **group**: categorical vector or factor giving group membership of columns of y. At least two categories need to be presented.

- **groupOrder**: an integer matrix with each row giving group indexes ordered based on decreasing expression levels.

- **M**: an integer indicating the number of groups being permutated. The range is \([2, K]\), where K is the total number of groups.

- **weights**: optional numeric matrix containing prior weights for each spot.

- **alpha**: parameter specifying within-group variance estimator to be used. ‘moderated’: empirical Bayes moderated variance estimator as used in `eBayes`. Numeric value: a constant value added to pooled variance estimator \((\alpha + \sigma)\). NULL: no estimator; all variances are set to be 1.

- **NumPerm**: an integer specifying the number of permutation resamplings (default 999).

- **seed**: an integer seed for the random number generator.

- **BPPARAM**: a BiocParallelParam object indicating whether parallelization should be used for permutation resamplings. The default is `bpparam()`.
pairwise_tstat_unscaled

Details

Top $M$ expressed groups will be involved in permutation. There are $\binom{M}{K}$ probe patterns in which probes are highly expressed in certain $M$ groups among the total $K$ groups. Probes of the same pattern share the same shuffled labels.

To improve the time efficiency, some functions within permutation loops are implemented using C++.

Value

a list containing the following components:

- `tstat.perm` a numeric matrix with each column giving OVESEG-test statistics over the expressions after one permutation.
- `topidx.perm` a integer matrix with each column giving the highest expressed group index over the expressions after one permutation.

Examples

data(RocheBT)
ppnull <- postProbNull(RocheBT$y, RocheBT$group, detail.return = TRUE)
rperm <- OVEtstatPermTopM(RocheBT$y, RocheBT$group, ppnull$groupOrder, M = 2, 
                      NumPerm = 99, BPPARAM = BiocParallel::SerialParam())

## Not run:
# parallel computing
rperm <- OVEtstatPermTopM(RocheBT$y, RocheBT$group, ppnull$groupOrder, M = 2, 
                      NumPerm = 99, BPPARAM = BiocParallel::SnowParam())

## End(Not run)

pairwise_tstat_unscaled

pairwise t-statistics (unscaled)

Description

pairwise t-statistics (unscaled)

Usage

pairwise_tstat_unscaled(ymean, stdevUnscaled)

Arguments

ymean a numeric matrix containing group means.
stdevUnscaled a numeric matrix containing unscaled standard deviations of the group means.

Value

unscaled pairwise t-statistics
patternDistri

Probabilities of all upregulation patterns

Description
This function estimates probabilities of all kinds of upregulation patterns among subtypes.

Usage
patternDistri(ppnull)

Arguments
ppnull a list returned by `postProbNull` or `OVESEGtest`.

Details
The probability of each upregulation pattern is calculated by accumulating and normalizing gene-wise posterior probability of null hypotheses and of alternative hypotheses.

Value
a data frame object containing all possible upregulation patterns and corresponding probabilities.

Examples
data(RocheBT)
ppnull <- postProbNull(RocheBT$y, RocheBT$group, alpha='moderated')
pd <- patternDistri(ppnull)

permfunc

Internal function for one permutation task

Description
Internal function for one permutation task

Usage
permfunc(p, y, group, weights, alpha, combM, geneSubset, seeds)
**Arguments**

- `p`: an integer indicating permutation index
- `y`: an expressions matrix
- `group`: a integer vector indicating group labels
- `weights`: optional numeric matrix containing prior weights
- `alpha`: parameter specifying within-group variance estimator to be used
- `combM`: a integer matrix with each row giving one choice of M groups
- `geneSubset`: a integer vector indicating the probe pattern of combM
- `seed`: an integer seed for the random number generator

**Value**

test statistics and upregulated group indexes after one permutation

---

**Description**

This function computes posterior probabilities of each component null hypothesis given observed probes. Such probe-wise probabilities will be used as weights for aggregating permutations.

**Usage**

```r
postProbNull(y, group, weights = NULL, alpha = "moderated", detail.return = TRUE)
```

**Arguments**

- `y`: a numeric matrix containing log-expression or logCPM (log2-counts per million) values. Data frame or SummarizedExperiment object will be internally coerced into a matrix. Rows correspond to probes and columns to samples. Missing values are not permitted.
- `group`: categorical vector or factor giving group membership of columns of `y`. At least two categories need to be presented.
- `weights`: optional numeric matrix containing prior weights for each spot.
- `alpha`: parameter specifying within-group variance estimator to be used. "moderated": empirical Bayes moderated variance estimator as used in eBayes. Numeric value: a constant value added to pooled variance estimator (α + σ). NULL: no estimator; all variances are set to be 1.
- `detail.return`: a logical indicating whether more details (e.g. lfdr) will be returned.
Details

Posterior probabilities of each component null hypothesis given observed probes are estimated from ANOVA test on certain groups and local fdr. There are totally \((K - 1)\) null hypotheses, where \(K\) is the number of groups.

Value

A list containing the following components:

- \(W\): A matrix of posterior probabilities for each component null hypothesis given an observed probe. Rows correspond to probes and columns to one hypothesis.
- \(label\): A vector of group labels.
- \(groupOrder\): A matrix with each row being group indexes ordered based on decreasing expression levels. Group indexes are positions in \(label\).
- \(F.p.value\): A matrix with each column giving p-values corresponding to F-statistics on certain groups.
- \(lfdr\): A matrix with each column being local false discovery rates estimated based on one column of weighted \(F.p.value\) matrix.
- \(fit\): A MArrayLM fitted model object produced by \(lmFit\).

\(F.p.value\), \(lfdr\) and \(fit\) are returned only when \(detail.return\) is TRUE.

Examples

```r
data(RocheBT)
ppnull <- postProbNull(RocheBT$y, RocheBT$group, alpha='moderated')
ppnull <- postProbNull(RocheBT$y, RocheBT$group, alpha=0.1)
```

### Description

This function estimates p-values by aggregating weighted permutations.

### Usage

```r
pvalueWeightedEst(tt, ttperm, w)
```

### Arguments

- **tt**: A vector of test statistics.
- **ttperm**: A matrix of test statistics from permutations. Rows correspond to probes and columns to one permutation.
- **w**: A matrix containing weights for each spot in \(ttperm\). Provided by \texttt{postProbNull}.
Details

P-values are estimated by weightedly accumulating test statistics from permutations that are larger than observations.

Value

p-values.

Examples

```r
# generate some example data
t.obs <- rnorm(100)
t.perm <- matrix(rnorm(100*1000),nrow=100)
w <- matrix(runif(100*1000),nrow=100)

tests <- pvalueWeightedEst(t.obs, t.perm, w)
```

Description

Three cell subtypes (B cells, CD4+ T cells, CD8+ T cells) were isolated from 5 pools of 5 healthy donors each. RNA obtained from these 15 purified populations were subsequently used for mRNA expression profiling by HG-U133Plus2.0 microarrays. We downsample the original data to 5000 probes/probesets. Subtype labels for purified populations are also included. (Data generation script can be found in `./data_raw` folder.)

Usage

```
data(RocheBT)
```

Format

A list with one expression matrix (y) and a categorical vector giving subtypes (group).

References

### row_min

<table>
<thead>
<tr>
<th>row_min</th>
<th>min value for each row</th>
</tr>
</thead>
</table>

**Description**

min value for each row

**Usage**

\[
\text{row\_min}(Y)
\]

**Arguments**

\[
Y \quad \text{a numeric matrix}
\]

**Value**

a numeric vector indicating min value in each row

---

### row_which_max

<table>
<thead>
<tr>
<th>row_which_max</th>
<th>which.max for each row</th>
</tr>
</thead>
</table>

**Description**

which.max for each row

**Usage**

\[
\text{row\_which\_max}(Y)
\]

**Arguments**

\[
Y \quad \text{a numeric matrix}
\]

**Value**

a integer vector indicating the location of max value in each row
shuffe_topm  

**shuffe_topm**  
**Shuffle the top M groups**

**Description**
Shuffle the top M groups

**Usage**

```
shuffe_topm(y, group, weights, combM, geneSubset, seed)
```

**Arguments**

- **y**: a numeric matrix to be shuffled.
- **group**: a integer vector indicating group indexes.
- **weights**: optional numeric matrix containing prior weights.
- **combM**: a integer matrix with each row giving one choice of M groups.
- **geneSubset**: a integer vector indicating the probe pattern of combM.
- **seed**: an integer seed for the random number generator.

**Value**

shuffled expression matrix and weight matrix in top M groups.
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