

Package ‘coseq’

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Description Co-expression analysis for expression profiles arising from high-throughput sequencing data. Feature (e.g., gene) profiles are clustered using adapted transformations and mixture models or a K-means algorithm, and model selection criteria (to choose an appropriate number of clusters) are provided.

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coseq-package	<i>Co-expression and co-abundance analysis of high-throughput sequencing data</i>
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Description

Co-expression analysis for expression profiles arising from high-throughput sequencing data. Feature (e.g., gene) profiles are clustered using adapted transformations and mixture models or a K-means algorithm, and model selection criteria (to choose an appropriate number of clusters) are provided.

Details

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Author(s)

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References

Godichon-Baggioni, A., Maugis-Rabusseau, C. and Rau, A. (2018) Clustering transformed compositional data using K-means, with applications in gene expression and bicycle sharing system data. *Journal of Applied Statistics*, doi:10.1080/02664763.2018.1454894.

Rau, A. and Maugis-Rabusseau, C. (2018) Transformation and model choice for co-expression analysis of RNA-seq data. *Briefings in Bioinformatics*, 19(3)-425-436.

Rau, A., Maugis-Rabusseau, C., Martin-Magniette, M.-L., Celeux, G. (2015) Co-expression analysis of high-throughput transcriptome sequencing data with Poisson mixture models. *Bioinformatics*, doi: 10.1093/bioinformatics/btu845.

Rau, A., Celeux, G., Martin-Magniette, M.-L., Maugis-Rabusseau, C. (2011) Clustering high-throughput sequencing data with Poisson mixture models. Inria Research Report 7786. Available at <http://hal.inria.fr/inria-00638082>.

Examples

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3,4
run_arcsin <- coseq(object=countmat, K=2:4, iter=5, transformation="arcsin",
                  model="Normal", seed=12345)

run_arcsin

## Plot and summarize results
plot(run_arcsin)
summary(run_arcsin)

## Compare ARI values for all models (no plot generated here)
ARI <- compareARI(run_arcsin, plot=FALSE)

## Compare ICL values for models with arcsin and logit transformations
run_logit <- coseq(object=countmat, K=2:4, iter=5, transformation="logit",
                  model="Normal")
compareICL(list(run_arcsin, run_logit))

## Use accessor functions to explore results
clusters(run_arcsin)
likelihood(run_arcsin)
nbCluster(run_arcsin)
ICL(run_arcsin)

## Examine transformed counts and profiles used for graphing
```

```

tcounts(run_arcsin)
profiles(run_arcsin)

## Run the K-means algorithm for logclr profiles for K = 2,..., 20
run_kmeans <- coseq(object=countmat, K=2:20, transformation="logclr",
                    model="kmeans")

run_kmeans

```

clusterEntropy	<i>Calculation of per-cluster entropy</i>
----------------	---

Description

Provides the calculation of per-cluster entropy, equivalent to

$$Entropy(k) = \sum_{i \in C_k} \log(\tau_{ik})$$

where τ_{ik} is the conditional probability of gene i belonging to cluster k and C_k corresponds to the set of indices of genes attributed to cluster k .

Usage

```
clusterEntropy(probaPost)
```

Arguments

probaPost	Matrix containing the conditional probabilities of belonging to each cluster for all observations
-----------	---

Value

Entropy per cluster

Author(s)

Cathy Maugis-Rabusseau

Examples

```

## Generate artificial matrix of conditional probabilities for K=5 clusters
tmp <- matrix(runif(100*5), nrow=100, ncol=5)
probaPost <- tmp / rowSums(tmp)
clusterEntropy(probaPost)

```

clusterInertia *Calculation of within-cluster inertia*

Description

Provides the calculation of within-cluster inertia, equivalent to

$$Inertia(k) = \sum_{i \in C_k} (y_{ik} - \mu_k)^2$$

where μ_k is the mean of cluster k and C_k corresponds to the set of indices of genes attributed to cluster k .

Usage

```
clusterInertia(profiles, clusters)
```

Arguments

profiles	Matrix, data.frame, or DataFrame containing the (transformed) profiles used for the clustering
clusters	Vector of cluster labels corresponding to the observations in profiles

Value

Within cluster inertia

Author(s)

Andrea Rau, Antoine Godichon-Baggioni

Examples

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
conds <- rep(c("A","B","C","D"), each=2)

## Run the K-means algorithm for logclr profiles for K = 2,..., 20
run_kmeans <- coseq(object=countmat, K=2:20, transformation="logclr",
model="kmeans")
clusterInertia(profiles=tcounts(run_kmeans), clusters=clusters(run_kmeans))
```

compareARI	<i>Pairwise comparisons of ARI values among a set of clustering partitions</i>
------------	--

Description

Provides the adjusted rand index (ARI) between pairs of clustering partitions.

Usage

```
compareARI(object, ...)
```

```
## S4 method for signature 'coseqResults'
```

```
compareARI(
  object,
  K = NULL,
  parallel = FALSE,
  BPPARAM = bpparam(),
  plot = TRUE,
  ...
)
```

```
## S4 method for signature 'matrix'
```

```
compareARI(object, parallel = FALSE, BPPARAM = bpparam(), plot = TRUE, ...)
```

```
## S4 method for signature 'data.frame'
```

```
compareARI(object, parallel = FALSE, BPPARAM = bpparam(), plot = TRUE, ...)
```

Arguments

object	Object of class <code>coseqResults</code> or <code>RangedSummarizedExperiment</code> , or alternatively a $n \times M$ <code>data.frame</code> or <code>matrix</code> containing the clustering partitions for M different models
...	Additional optional parameters for <code>corrplot</code>
K	If <code>NULL</code> , pairwise ARI values will be calculated among every model in object <code>x</code> . Otherwise, <code>K</code> provides a vector of cluster numbers identifying a subset of models in <code>x</code> .
parallel	If <code>FALSE</code> , no parallelization. If <code>TRUE</code> , parallel execution using <code>BiocParallel</code> (see next argument <code>BPPARAM</code>). Note that parallelization is unlikely to be helpful unless the number of observations n in the clustering partitions or the number of models M are very large.
BPPARAM	Optional parameter object passed internally to <code>bplapply</code> when <code>parallel=TRUE</code> . If not specified, the parameters last registered with <code>register</code> will be used.
plot	If <code>TRUE</code> , provide a heatmap using <code>corrplot</code> to visualize the calculated pairwise ARI values.

Value

Matrix of adjusted rand index values calculated between each pair of models.

Author(s)

Andrea Rau

Examples

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3,4
run_arcsin <- coseq(object=countmat, K=2:4, iter=5, transformation="arcsin",
                  model="Normal", seed=12345)
run_arcsin

## Plot and summarize results
plot(run_arcsin)
summary(run_arcsin)

## Compare ARI values for all models (no plot generated here)
ARI <- compareARI(run_arcsin, plot=FALSE)

## Compare ICL values for models with arcsin and logit transformations
run_logit <- coseq(object=countmat, K=2:4, iter=5, transformation="logit",
                  model="Normal")
compareICL(list(run_arcsin, run_logit))

## Use accessor functions to explore results
clusters(run_arcsin)
likelihood(run_arcsin)
nbCluster(run_arcsin)
ICL(run_arcsin)

## Examine transformed counts and profiles used for graphing
tcounts(run_arcsin)
profiles(run_arcsin)

## Run the K-means algorithm for logclr profiles for K = 2,..., 20
run_kmeans <- coseq(object=countmat, K=2:20, transformation="logclr",
                  model="kmeans")
run_kmeans
```

 compareICL

Compare corrected ICL values after data transformation

Description

Compare the corrected ICL values after applying the arcsin, logit, and logMedianRef transformations in a coseq analysis

Usage

```
compareICL(x)
```

Arguments

x A list made up of coseqResults objects. At the current time, this function only supports the comparison of coseqResults objects using model="Normal" and transformation = c("arcsin", "logit", "logMedianRef")

Value

A plot of corrected ICL values for the models included in x (the list of coseqResults objects)

Author(s)

Andrea Rau, Cathy Maugis-Rabusseau

Examples

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3,4
run_arcsin <- coseq(object=countmat, K=2:4, iter=5, transformation="arcsin",
                  model="Normal", seed=12345)
run_arcsin

## Plot and summarize results
plot(run_arcsin)
summary(run_arcsin)

## Compare ARI values for all models (no plot generated here)
ARI <- compareARI(run_arcsin, plot=FALSE)

## Compare ICL values for models with arcsin and logit transformations
run_logit <- coseq(object=countmat, K=2:4, iter=5, transformation="logit",
                  model="Normal")
```



```
compareICL(list(run_arcsin, run_logit))

## Use accessor functions to explore results
clusters(run_arcsin)
likelihood(run_arcsin)
nbCluster(run_arcsin)
ICL(run_arcsin)

## Examine transformed counts and profiles used for graphing
tcounts(run_arcsin)
profiles(run_arcsin)

## Run the K-means algorithm for logclr profiles for K = 2,..., 20
run_kmeans <- coseq(object=countmat, K=2:20, transformation="logclr",
                    model="kmeans")
run_kmeans
```

convertLegacyCoseq *Convert legacy coseq objects*

Description

Convert legacy coseq S3 class objects to coseqResults S4 class objects

Usage

```
convertLegacyCoseq(object, digits = 3)
```

Arguments

object	Object of S3 class coseq arising from a call to previous versions of coseq (< 0.99.1)
digits	integer indicating the number of decimal places (round) to retain in results.

Value

Converted object of S4 class coseqResults compatible with recent versions of coseq ($\geq 0.99.1$)

coseq	<i>Co-expression or co-abundance analysis of high-throughput sequencing data</i>
-------	--

Description

This is the primary user interface for the coseq package. Generic S4 methods are implemented to perform co-expression or co-abundance analysis of high-throughput sequencing data, with or without data transformation, using K-means or mixture models. The supported classes are `matrix`, `data.frame`, and `DESeqDataSet`. The output of coseq is an S4 object of class `coseqResults`.

Usage

```
coseq(object, ...)
```

```
## S4 method for signature 'matrix'
```

```
coseq(
  object,
  K,
  subset = NULL,
  model = "kmeans",
  transformation = "logclr",
  normFactors = "TMM",
  meanFilterCutoff = NULL,
  modelChoice = ifelse(model == "kmeans", "DDSE", "ICL"),
  parallel = FALSE,
  BPPARAM = bpparam(),
  seed = NULL,
  ...
)
```

```
## S4 method for signature 'data.frame'
```

```
coseq(
  object,
  K,
  subset = NULL,
  model = "kmeans",
  transformation = "logclr",
  normFactors = "TMM",
  meanFilterCutoff = NULL,
  modelChoice = ifelse(model == "kmeans", "DDSE", "ICL"),
  parallel = FALSE,
  BPPARAM = bpparam(),
  seed = NULL,
  ...
)
```

```
## S4 method for signature 'DESeqDataSet'
coseq(
  object,
  K,
  model = "kmeans",
  transformation = "logclr",
  normFactors = "TMM",
  meanFilterCutoff = NULL,
  modelChoice = ifelse(model == "kmeans", "DDSE", "ICL"),
  parallel = FALSE,
  BPPARAM = bpparam(),
  seed = NULL,
  ...
)
```

Arguments

object	Data to be clustered. May be provided as a y ($n \times q$) matrix or data.frame of observed counts for n observations and q variables, or an object of class DESeqDataSet arising from a differential analysis via DESeq2.
...	Additional optional parameters.
K	Number of clusters (a single value or a vector of values)
subset	Optional vector providing the indices of a subset of genes that should be used for the co-expression analysis (i.e., row indices of the data matrix y). For the generic function coseq, the results of a previously run differential analysis may be used to select a subset of genes on which to perform the co-expression analysis. If this is desired, subset.index can also be an object of class DESeqResults (from the results function in DESeq2).
model	Type of mixture model to use ("Poisson" or "Normal"), or alternatively "kmeans" for a K-means algorithm
transformation	Transformation type to be used: "voom", "logRPKM" (if geneLength is provided by user), "arcsin", "logit", "logMedianRef", "profile", "logclr", "clr", "alr", "ilr", or "none"
normFactors	The type of estimator to be used to normalize for differences in library size: ("TC" for total count, "UQ" for upper quantile, "Med" for median, "DESeq" for the normalization method in the DESeq package, and "TMM" for the TMM normalization method (Robinson and Oshlack, 2010). Can also be a vector (of length q) containing pre-estimated library size estimates for each sample, or "none" if no normalization is required.
meanFilterCutoff	Value used to filter low mean normalized counts if desired (by default, set to a value of 50)
modelChoice	Criterion used to select the best model. For Gaussian mixture models, "ICL" (integrated completed likelihood criterion) is currently supported. For Poisson mixture models, "ICL", "BIC" (Bayesian information criterion), and a non-asymptotic criterion calibrated via the slope heuristics using either the "DDSE"

	(data-driven slope estimation) or “Djump” (dimension jump) approaches may be used. See the HTSCluster package documentation for more details about the slope heuristics approaches.
parallel	If FALSE, no parallelization. If TRUE, parallel execution using BiocParallel (see next argument BPPARAM). A note on running in parallel using BiocParallel: it may be advantageous to remove large, unneeded objects from the current R environment before calling the function, as it is possible that R’s internal garbage collection will copy these files while running on worker nodes.
BPPARAM	Optional parameter object passed internally to bplapply when parallel=TRUE. If not specified, the parameters last registered with register will be used.
seed	If desired, an integer defining the seed of the random number generator. If NULL, a random seed is used.

Value

An S4 object of class `coseqResults`, where conditional probabilities of cluster membership for each gene in each model is stored as a SimpleList of assay data, and the corresponding log likelihood, ICL value, number of clusters, and form of Gaussian model for each model are stored as metadata.

Author(s)

Andrea Rau

Examples

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3,4
run_arcsin <- coseq(object=countmat, K=2:4, iter=5, transformation="arcsin",
                  model="Normal", seed=12345)
run_arcsin

## Plot and summarize results
plot(run_arcsin)
summary(run_arcsin)

## Compare ARI values for all models (no plot generated here)
ARI <- compareARI(run_arcsin, plot=FALSE)

## Compare ICL values for models with arcsin and logit transformations
run_logit <- coseq(object=countmat, K=2:4, iter=5, transformation="logit",
                  model="Normal")
compareICL(list(run_arcsin, run_logit))

## Use accessor functions to explore results
```

```
clusters(run_arcsin)
likelihood(run_arcsin)
nbCluster(run_arcsin)
ICL(run_arcsin)

## Examine transformed counts and profiles used for graphing
tcounts(run_arcsin)
profiles(run_arcsin)

## Run the K-means algorithm for logclr profiles for K = 2,..., 20
run_kmeans <- coseq(object=countmat, K=2:20, transformation="logclr",
                    model="kmeans")

run_kmeans
```

coseqFullResults *Accessors for the assigned cluster labels of a coseqResults object.*

Description

The counts slot holds the count data as a matrix of non-negative integer count values, one row for each observational unit (gene or the like), and one column for each sample.

Usage

```
coseqFullResults(object, ...)

clusters(object, ...)

likelihood(object, ...)

nbCluster(object, ...)

proba(object, ...)

ICL(object, ...)

profiles(object, ...)

tcounts(object, ...)

transformationType(object, ...)

model(object, ...)

DDSEextract(object, ...)

Djumpextract(object, ...)
```

```
## S4 method for signature 'coseqResults'  
clusters(object, K)  
  
## S4 method for signature 'RangedSummarizedExperiment'  
clusters(object, ...)  
  
## S4 method for signature 'matrix'  
clusters(object, ...)  
  
## S4 method for signature 'data.frame'  
clusters(object, ...)  
  
## S4 method for signature 'MixmodCluster'  
likelihood(object)  
  
## S4 method for signature 'RangedSummarizedExperiment'  
likelihood(object)  
  
## S4 method for signature 'coseqResults'  
likelihood(object)  
  
## S4 method for signature ``NULL``  
likelihood(object)  
  
## S4 method for signature 'MixmodCluster'  
nbCluster(object)  
  
## S4 method for signature 'RangedSummarizedExperiment'  
nbCluster(object)  
  
## S4 method for signature 'coseqResults'  
nbCluster(object)  
  
## S4 method for signature ``NULL``  
nbCluster(object)  
  
## S4 method for signature 'MixmodCluster'  
ICL(object)  
  
## S4 method for signature 'RangedSummarizedExperiment'  
ICL(object)  
  
## S4 method for signature 'coseqResults'  
ICL(object)  
  
## S4 method for signature ``NULL``  
ICL(object)
```

```
## S4 method for signature 'coseqResults'  
profiles(object)  
  
## S4 method for signature 'coseqResults'  
tcounts(object)  
  
## S4 method for signature 'coseqResults'  
transformationType(object)  
  
## S4 method for signature 'coseqResults'  
model(object)  
  
## S4 method for signature 'coseqResults'  
coseqFullResults(object)  
  
## S4 method for signature 'coseqResults'  
show(object)  
  
## S4 method for signature 'MixmodCluster'  
proba(object)  
  
## S4 method for signature 'Capushe'  
DDSEextract(object)  
  
## S4 method for signature 'Capushe'  
Djumpextract(object)
```

Arguments

object	a coseqResults, RangedSummarizedExperiment, or MixmodCluster object.
...	Additional optional parameters
K	numeric indicating the model to be used (if NULL or missing, the model chosen by ICL is used by default)

Value

Output varies depending on the method. clusters returns a vector of cluster labels for each gene for the desired model.

Author(s)

Andrea Rau

Examples

```
## Simulate toy data, n = 300 observations  
set.seed(12345)  
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)  
countmat <- countmat[which(rowSums(countmat) > 0),]
```

```

conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3,4
run_arcsin <- coseq(object=countmat, K=2:4, iter=5, transformation="arcsin",
                   model="Normal", seed=12345)
run_arcsin

## Plot and summarize results
plot(run_arcsin)
summary(run_arcsin)

## Compare ARI values for all models (no plot generated here)
ARI <- compareARI(run_arcsin, plot=FALSE)

## Compare ICL values for models with arcsin and logit transformations
run_logit <- coseq(object=countmat, K=2:4, iter=5, transformation="logit",
                  model="Normal")
compareICL(list(run_arcsin, run_logit))

## Use accessor functions to explore results
clusters(run_arcsin)
likelihood(run_arcsin)
nbCluster(run_arcsin)
ICL(run_arcsin)

## Examine transformed counts and profiles used for graphing
tcounts(run_arcsin)
profiles(run_arcsin)

## Run the K-means algorithm for logclr profiles for K = 2,..., 20
run_kmeans <- coseq(object=countmat, K=2:20, transformation="logclr",
                   model="kmeans")
run_kmeans

```

coseqResults-class *coseqResults* object and constructor

Description

coseqResults is a subclass of RangedSummarizedExperiment, used to store the co-expression results as well as some additional information useful for plotting (tcounts, y_profiles) and meta-information about the co-expression analysis (transformation, normFactors).

Usage

```

coseqResults(
  SummarizedExperiment,
  allResults,
  model = NULL,
  transformation = NULL,

```



```

    tcounts = NULL,
    y_profiles = NULL,
    normFactors = NULL
  )

```

Arguments

SummarizedExperiment	a RangedSummarizedExperiment of coseq results
allResults	List of conditional probabilities of cluster membership for each gene, in all models fit
model	"Normal" or "Poisson", the mixture model used for co-expression
transformation	Transformation applied to counts to obtain tcounts
tcounts	Transformed counts used for mixture model fitting
y_profiles	y profiles used for coseq plotting
normFactors	Scaling factors used for normalization

Details

This constructor function would not typically be used by "end users". This simple class extends the RangedSummarizedExperiment class of the SummarizedExperiment package to allow other packages to write methods for results objects from the coseq package. It is used by `coseqRun` to wrap up the results table.

Value

a coseqResults object

coseqRun	<i>Co-expression analysis</i>
----------	-------------------------------

Description

Function for primary code to perform co-expression analysis, with or without data transformation, using mixture models. The output of `coseqRun` is an S4 object of class `coseqResults`.

Usage

```

coseqRun(
  y,
  K,
  conds = NULL,
  normFactors = "TMM",
  model = "kmeans",
  transformation = "logclr",
  subset = NULL,

```

```

meanFilterCutoff = 50,
modelChoice = ifelse(model == "kmeans", "DDSE", "ICL"),
parallel = FALSE,
BPPARAM = bpparam(),
seed = NULL,
...
)

```

Arguments

<code>y</code>	$(n \times q)$ matrix of observed counts for n observations (genes) and q variables (samples). In nearly all cases, $n > q$.
<code>K</code>	Number of clusters (a single value or a vector of values)
<code>conds</code>	Vector of length q defining the condition (treatment group) for each variable (column) in <code>y</code>
<code>normFactors</code>	The type of estimator to be used to normalize for differences in library size: (“TC” for total count, “UQ” for upper quantile, “Med” for median, “DESeq” for the normalization method in the DESeq package, and “TMM” for the TMM normalization method (Robinson and Oshlack, 2010). Can also be a vector (of length q) containing pre-estimated library size estimates for each sample, or “none” if no normalization is required.
<code>model</code>	Type of mixture model to use (“Poisson” or “Normal”), or alternatively “kmeans” for a K-means algorithm
<code>transformation</code>	Transformation type to be used: “voom”, “logRPKM” (if <code>geneLength</code> is provided by user), “arcsin”, “logit”, “logMedianRef”, “profile”, “logclr”, “clr”, “alr”, “ilr”, or “none”
<code>subset</code>	Optional vector providing the indices of a subset of genes that should be used for the co-expression analysis (i.e., row indices of the data matrix <code>y</code>). For the generic function <code>coseq</code> , the results of a previously run differential analysis may be used to select a subset of genes on which to perform the co-expression analysis. If this is desired, <code>subset.index</code> can also be an object of class <code>DESeqResults</code> (from the <code>results</code> function in <code>DESeq2</code>).
<code>meanFilterCutoff</code>	Value used to filter low mean normalized counts if desired (by default, set to a value of 50)
<code>modelChoice</code>	Criterion used to select the best model. For Gaussian mixture models, “ICL” (integrated completed likelihood criterion) is currently supported. For Poisson mixture models, “ICL”, “BIC” (Bayesian information criterion), and a non-asymptotic criterion calibrated via the slope heuristics using either the “DDSE” (data-driven slope estimation) or “Djump” (dimension jump) approaches may be used. See the <code>HTScluster</code> package documentation for more details about the slope heuristics approaches.
<code>parallel</code>	If <code>FALSE</code> , no parallelization. If <code>TRUE</code> , parallel execution using <code>BiocParallel</code> (see next argument <code>BPPARAM</code>). A note on running in parallel using <code>BiocParallel</code> : it may be advantageous to remove large, unneeded objects from the current R environment before calling the function, as it is possible that R’s internal garbage collection will copy these files while running on worker nodes.

BPPARAM	Optional parameter object passed internally to <code>bplapply</code> when <code>parallel=TRUE</code> . If not specified, the parameters last registered with <code>register</code> will be used.
seed	If desired, an integer defining the seed of the random number generator. If <code>NULL</code> , a random seed is used.
...	Additional optional parameters.

Value

An S4 object of class `coseqResults` whose assays contain a `SimpleList` object, where each element in the list corresponds to the conditional probabilities of cluster membership for each gene in each model. Meta data (accessible via `metatdata` include the model used (either `Normal` or `Poisson`), the transformation used on the data, the transformed data using to estimate model (`tcounts`), the normalized profiles for use in plotting (`y_profiles`), and the normalization factors used in the analysis (`normFactors`).

Author(s)

Andrea Rau

Examples

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
conds <- rep(c("A","B","C","D"), each=2)

## Run the K-means for K = 2,3,4 with logCLR transformation
## The following are equivalent:
run <- coseqRun(y=countmat, K=2:15)
run <- coseq(object=countmat, K=2:15, transformation="logclr", model="kmeans")

## Run the Normal mixture model for K = 2,3,4 with arcsine transformation
## The following are equivalent:
run <- coseqRun(y=countmat, K=2:4, iter=5, transformation="arcsin", model="Normal")
run <- coseq(object=countmat, K=2:4, iter=5, transformation="arcsin", model="Normal")
```

fietz

RNA-seq data from the mouse neocortex in Fietz et al. (2012)

Description

This dataset represents RNA-seq data from mouse neocortex RNA-seq data in five embryonic (day 14.5) mice by analyzing the transcriptome of three regions: the ventricular zone (VZ), subventricular zone (SVZ) and cortical plate (CP).

Usage

```
data(fietz)
```

Format

An ExpressionSet named `fietz.eset` containing the phenotype data and expression data for the Fietz et al. (2012) experiment. Phenotype data may be accessed using the `pData` function, and expression data may be accessed using the `exprs` function.

Value

Object of class 'ExpressionSet'. Matrix of counts can be accessed after loading the 'Biobase' package and calling `exprs(fietz)`.

Source

Digital Expression Explorer (<http://dee.bakeridi.edu.au/>).

References

<https://perso.math.univ-toulouse.fr/maugis/mixstatseq/packages>

Fietz, S. A., et al. (2012). Transcriptomes of germinal zones of human and mouse fetal neocortex suggest a role of extracellular matrix in progenitor self-renewal. *Proceedings of the National Academy of Sciences*, 109(29):11836-11841.

kmeansProbaPost	<i>Calculate conditional probabilities of cluster membership for K-means clustering</i>
-----------------	---

Description

Calculate conditional probabilities of cluster membership for K-means clustering

Usage

```
kmeansProbaPost(clusters, tcounts)
```

Arguments

<code>clusters</code>	Cluster labels arising from K-means clustering
<code>tcounts</code>	Transformed counts clustered using K-means

Value

Conditional probabilities of cluster membership for each observation in each cluster

Examples

```
## Example of K-means taken from ?kmeans help page
x <- rbind(matrix(rnorm(100, sd = 0.3), ncol = 2),
            matrix(rnorm(100, mean = 1, sd = 0.3), ncol = 2))
colnames(x) <- c("x", "y")
cl <- kmeans(x, 5)
probaPost <- kmeansProbaPost(cl$cluster, x)
head(probaPost)
```

logclr

Calculate the Log Centered Log Ratio (logCLR) transformation

Description

Calculate the Log Centered Log Ratio (logCLR) transformation

Usage

```
logclr(profiles)
```

Arguments

profiles Matrix of profiles. Note that the presence of 0 values causes an error message to be produced.

Value

logCLR-transformed profiles

matchContTable

Permute columns of a contingency table

Description

Permute the columns of a contingency table comparing two clusterings to load the diagonal as much as possible.

Usage

```
matchContTable(table_1, table_2)
```

Arguments

table_1 Partition from a first data clustering
table_2 Partition from a second data clustering

Value

Permuted contingency table

Examples

```
## Generate arbitrary labels from two separate clustering results
labels_1 <- sample(1:10, 1000, replace=TRUE) ## K=10 clusters
labels_2 <- sample(1:8, 1000, replace=TRUE)  ## K=8 clusters
matchContTable(labels_1, labels_2)
```

NormMixClus	<i>Normal mixture model estimation and selection for a series of cluster numbers</i>
-------------	--

Description

Perform co-expression and co-abundance analysis of high-throughput sequencing data, with or without data transformation, using a Normal mixture models. The output of NormMixClus is an S4 object of class RangedSummarizedExperiment.

Usage

```
NormMixClus(
  y_profiles,
  K,
  subset = NULL,
  parallel = TRUE,
  BPPARAM = bpparam(),
  seed = NULL,
  ...
)
```

Arguments

<code>y_profiles</code>	$(n \times q)$ matrix of observed profiles for n observations and q variables
<code>K</code>	Number of clusters (a single value or a sequence of values).
<code>subset</code>	Optional vector providing the indices of a subset of genes that should be used for the co-expression analysis (i.e., row indices of the data matrix y).
<code>parallel</code>	If FALSE, no parallelization. If TRUE, parallel execution using BiocParallel (see next argument BPPARAM). A note on running in parallel using BiocParallel: it may be advantageous to remove large, unneeded objects from the current R environment before calling the function, as it is possible that R's internal garbage collection will copy these files while running on worker nodes.
<code>BPPARAM</code>	Optional parameter object passed internally to <code>bplapply</code> when <code>parallel=TRUE</code> . If not specified, the parameters last registered with <code>register</code> will be used.

seed If desired, an integer defining the seed of the random number generator. If NULL, a random seed is used.

... Additional optional parameters to be passed to [NormMixClusK](#).

Value

An S4 object of class `coseqResults`, with conditional probabilities of cluster membership for each gene in each model stored as a list of assay data, and corresponding log likelihood, ICL value, number of clusters, and form of Gaussian model for each model stored as metadata.

Author(s)

Andrea Rau, Cathy Maugis-Rabusseau

Examples

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
profiles <- transformRNAseq(countmat, norm="none",
                           transformation="arcsin")$tcounts

conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3
## Object of class coseqResults
run <- NormMixClus(y=profiles, K=2:3, iter=5)
run

## Run the Normal mixture model for K=2
## Object of class SummarizedExperiment
run2 <- NormMixClusK(y=profiles, K=2, iter=5)

## Summary of results
summary(run)

## Re-estimate mixture parameters for the model with K=2 clusters
param <- NormMixParam(run, y_profiles=profiles)
```

NormMixClusK

Normal mixture model estimation

Description

Perform co-expression and co-abundance analysis of high-throughput sequencing data, with or without data transformation, using a Normal mixture models for single number of clusters K . The output of `NormMixClusK` is an S4 object of class `RangedSummarizedExperiment`.

Usage

```

NormMixClusK(
  y_profiles,
  K,
  alg.type = "EM",
  init.runs = 50,
  init.type = "small-em",
  GaussianModel = "Gaussian_pk_Lk_Ck",
  init.iter = 20,
  iter = 1000,
  cutoff = 0.001,
  verbose = TRUE,
  digits = 3,
  seed = NULL
)

```

Arguments

<code>y_profiles</code>	y ($n \times q$) matrix of observed profiles for n observations and q variables
<code>K</code>	Number of clusters (a single value).
<code>alg.type</code>	Algorithm to be used for parameter estimation: "EM", "CEM", "SEM"
<code>init.runs</code>	Number of runs to be used for the Small-EM strategy, with a default value of 50
<code>init.type</code>	Type of initialization strategy to be used: "small-em" for the Small-EM strategy, "random", "CEM", or "SEMMax"
<code>GaussianModel</code>	One of the 28 forms of Gaussian models defined in Rmixmod, by default equal to the "Gaussian_pk_Lk_Ck" (i.e., a general family model with free proportions, free volume, free shape, and free orientation)
<code>init.iter</code>	Number of iterations to be used within each run for the Small-EM strategy, with a default value of 20
<code>iter</code>	Maximum number of iterations to be run for the chosen algorithm
<code>cutoff</code>	Cutoff to declare algorithm convergence
<code>verbose</code>	If TRUE, verbose output is created
<code>digits</code>	Integer indicating the number of decimal places to be used for the probaPost output
<code>seed</code>	If desired, an integer defining the seed of the random number generator. If NULL, a random seed is used.

Value

An S4 object of class `RangedSummarizedExperiment`, with conditional probabilities of cluster membership for each gene stored as assay data, and log likelihood, ICL value, number of clusters, and form of Gaussian model stored as metadata.

Author(s)

Cathy Maugis-Rabusseau, Andrea Rau

Examples

```

## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
profiles <- transformRNAseq(countmat, norm="none",
                           transformation="arcsin")$tcounts

conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3
## Object of class coseqResults
run <- NormMixClus(y=profiles, K=2:3, iter=5)
run

## Run the Normal mixture model for K=2
## Object of class SummarizedExperiment
run2 <- NormMixClusK(y=profiles, K=2, iter=5)

## Summary of results
summary(run)

## Re-estimate mixture parameters for the model with K=2 clusters
param <- NormMixParam(run, y_profiles=profiles)

```

NormMixParam

Calculate the mean and covariance for a Normal mixture model

Description

Calculates the mean and covariance parameters for a normal mixture model of the form pK_Lk_Ck

Usage

```

NormMixParam(
  coseqResults,
  y_profiles = NULL,
  K = NULL,
  digits = 3,
  plot = FALSE,
  ...
)

```

Arguments

coseqResults Object of class coseqResults or RangedSummarizedExperiment (as output from the NormMixClus or NormMixClusK functions)

<code>y_profiles</code>	y ($n \times q$) matrix of observed profiles for n observations and q variables, required for x of class <code>RangedSummarizedExperiment</code>
<code>K</code>	The model used for parameter estimation for objects x of class <code>coseq</code> or <code>NormMixClus</code> . When <code>NULL</code> , the model selected by the ICL criterion is used; otherwise, K should designate the number of clusters in the desired model
<code>digits</code>	Integer indicating the number of decimal places to be used for output
<code>plot</code>	If true, produce heatmaps to visualize the estimated per-cluster correlation matrices
<code>...</code>	Additional optional parameters to pass to <code>corrplot</code> , if desired

Value

<code>pi</code>	Vector of dimension K with the estimated cluster proportions from the Gaussian mixture model, where K is the number of clusters
<code>mu</code>	Matrix of dimension $K \times d$ containing the estimated mean vector from the Gaussian mixture model, where d is the number of samples in the data <code>y_profiles</code> and K is the number of clusters
<code>Sigma</code>	Array of dimension $d \times d \times K$ containing the estimated covariance matrices from the Gaussian mixture model, where d is the number of samples in the data <code>y_profiles</code> and K is the number of clusters
<code>rho</code>	Array of dimension $d \times d \times K$ containing the estimated correlation matrices from the Gaussian mixture model, where d is the number of samples in the data <code>y_profiles</code> and K is the number of clusters

Author(s)

Andrea Rau, Cathy Maugis-Rabusseau

Examples

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
profiles <- transformRNAseq(countmat, norm="none",
                           transformation="arcsin")$tcounts

conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3
## Object of class coseqResults
run <- NormMixClus(y=profiles, K=2:3, iter=5)
run

## Run the Normal mixture model for K=2
## Object of class SummarizedExperiment0
run2 <- NormMixClusK(y=profiles, K=2, iter=5)

## Summary of results
```

```
summary(run)

## Re-estimate mixture parameters for the model with K=2 clusters
param <- NormMixParam(run, y_profiles=profiles)
```

plot

Visualize results from coseq clustering

Description

Plot a coseqResults object.

Usage

```
plot(x, ...)
```

S4 method for signature 'coseqResults'

```
plot(
  x,
  y_profiles = NULL,
  K = NULL,
  threshold = 0.8,
  conds = NULL,
  average_over_conds = FALSE,
  collapse_reps = "none",
  graphs = c("logLike", "ICL", "profiles", "boxplots", "probapost_boxplots",
    "probapost_barplots", "probapost_histogram"),
  order = FALSE,
  profiles_order = NULL,
  n_row = NULL,
  n_col = NULL,
  add_lines = TRUE,
  ...
)
```

```
coseqGlobalPlots(object, graphs = c("logLike", "ICL"), ...)
```

```
coseqModelPlots(
  probaPost,
  y_profiles,
  K = NULL,
  threshold = 0.8,
  conds = NULL,
  collapse_reps = "none",
  graphs = c("profiles", "boxplots", "probapost_boxplots", "probapost_barplots",
    "probapost_histogram"),
```

```

order = FALSE,
profiles_order = NULL,
n_row = NULL,
n_col = NULL,
add_lines = TRUE,
...
)

```

Arguments

x	An object of class "coseqResults"
...	Additional optional plotting arguments (e.g., xlab, ylab, use_sample_names, facet_labels)
y_profiles	y ($n \times q$) matrix of observed profiles for n observations and q variables to be used for graphing results (optional for logLike, ICL, probapost_boxplots, and probapost_barplots, and by default takes value x\$tcunts if NULL)
K	If desired, the specific model to use for plotting (or the specific cluster number(s) to use for plotting in the case of coseqModelPlots). If NULL, all clusters will be visualized, and the model chosen by ICL will be plotted
threshold	Threshold used for maximum conditional probability; only observations with maximum conditional probability greater than this threshold are visualized
conds	Condition labels, if desired
average_over_conds	If TRUE, average values of y_profiles within each condition identified by conds for the profiles and boxplots plots. This argument is redundant to collapse_reps = "sum", and collapse_reps should be used instead.
collapse_reps	If "none", display all replicates. If "sum", collapse replicates within each condition by summing their profiles. If "average", collapse replicates within each condition by averaging their profiles. For highly unbalanced experimental designs, using "average" will likely provide more easily interpretable plots.
graphs	Graphs to be produced, one (or more) of the following: "logLike" (log-likelihood plotted versus number of clusters), "ICL" (ICL plotted versus number of clusters), "profiles" (line plots of profiles in each cluster), "boxplots" (boxplots of profiles in each cluster), "probapost_boxplots" (boxplots of maximum conditional probabilities per cluster), "probapost_barplots" (number of observations with a maximum conditional probability greater than threshold per cluster), "probapost_histogram" (histogram of maximum conditional probabilities over all clusters)
order	If TRUE, order clusters in probapost_boxplot by median and probapost_barplot by number of observations with maximum conditional probability greater than threshold
profiles_order	If NULL or FALSE, line plots and boxplots of profiles are plotted sequentially by cluster number (K=1, K=2, ...). If TRUE, line plots and boxplots of profiles are plotted in an automatically calculated order (according to the Euclidean distance between cluster means) to plot clusters with similar mean profiles next to one another. Otherwise, the user may provide a vector (of length equal to the number of clusters in the given model) providing the desired order of plots.

n_row	Number of rows for plotting layout of line plots and boxplots of profiles.
n_col	Number of columns for plotting layout of line plots and boxplots of profiles.
add_lines	If TRUE, add red lines representing means to boxplots; if FALSE, these will be suppressed.
object	An object of class "RangedSummarizedExperiment" arising from a call to NormMixClus
probaPost	Matrix or data.frame of dimension ($n \times K$) containing the conditional probabilities of cluster membership for n genes in K clusters arising from a mixture model

Value

Named list of plots of the coseqResults object.

Author(s)

Andrea Rau, Cathy Maugis-Rabusseau

Examples

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3,4
run_arcsin <- coseq(object=countmat, K=2:4, iter=5, transformation="arcsin",
                  model="Normal", seed=12345)
run_arcsin

## Plot and summarize results
plot(run_arcsin)
summary(run_arcsin)

## Compare ARI values for all models (no plot generated here)
ARI <- compareARI(run_arcsin, plot=FALSE)

## Compare ICL values for models with arcsin and logit transformations
run_logit <- coseq(object=countmat, K=2:4, iter=5, transformation="logit",
                  model="Normal")
compareICL(list(run_arcsin, run_logit))

## Use accessor functions to explore results
clusters(run_arcsin)
likelihood(run_arcsin)
nbCluster(run_arcsin)
ICL(run_arcsin)

## Examine transformed counts and profiles used for graphing
tcounts(run_arcsin)
profiles(run_arcsin)
```

```
## Run the K-means algorithm for logclr profiles for K = 2,..., 20
run_kmeans <- coseq(object=countmat, K=2:20, transformation="logclr",
                    model="kmeans")
run_kmeans
```

```
summary,coseqResults-method
```

Summarize results from coseq clustering

Description

A function to summarize the clustering results obtained from a Poisson or Gaussian mixture model estimated using coseq. In particular, the function provides the number of clusters selected for the ICL model selection approach (or alternatively, for the capushe non-asymptotic approach if K-means clustering is used), number of genes assigned to each cluster, and if desired the per-gene cluster means.

Usage

```
## S4 method for signature 'coseqResults'
summary(object, y_profiles, digits = 3, ...)
```

Arguments

object	An object of class "coseqResults"
y_profiles	Data used for clustering if per-cluster means are desired
digits	Integer indicating the number of decimal places to be used for mixture model parameters
...	Additional arguments

Details

Provides the following summary of results:

- 1) Number of clusters and model selection criterion used, if applicable.
- 2) Number of observations across all clusters with a maximum conditional probability greater than 90 (observations) for the selected model.
- 3) Number of observations per cluster with a maximum conditional probability greater than 90 (cluster) for the selected model.
- 4) If desired, the μ values and π values for the selected model in the case of a Gaussian mixture model.

Value

Summary of the coseqResults object.

Author(s)

Andrea Rau

References

Rau, A. and Maugis-Rabusseau, C. (2017) Transformation and model choice for co-expression analysis of RNA-seq data. Briefings in Bioinformatics, doi: <http://dx.doi.org/10.1101/065607>.

Godichon-Baggioni, A., Maugis-Rabusseau, C. and Rau, A. (2017) Clustering transformed compositional data using K-means, with applications in gene expression and bicycle sharing system data. arXiv:1704.06150.

See Also[coseq](#)**Examples**

```
## Simulate toy data, n = 300 observations
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
conds <- rep(c("A","B","C","D"), each=2)

## Run the Normal mixture model for K = 2,3,4
run_arcsin <- coseq(object=countmat, K=2:4, iter=5, transformation="arcsin",
                  model="Normal", seed=12345)
run_arcsin

## Plot and summarize results
plot(run_arcsin)
summary(run_arcsin)

## Compare ARI values for all models (no plot generated here)
ARI <- compareARI(run_arcsin, plot=FALSE)

## Compare ICL values for models with arcsin and logit transformations
run_logit <- coseq(object=countmat, K=2:4, iter=5, transformation="logit",
                  model="Normal")
compareICL(list(run_arcsin, run_logit))

## Use accessor functions to explore results
clusters(run_arcsin)
likelihood(run_arcsin)
nbCluster(run_arcsin)
ICL(run_arcsin)

## Examine transformed counts and profiles used for graphing
tcounts(run_arcsin)
profiles(run_arcsin)

## Run the K-means algorithm for logclr profiles for K = 2,..., 20
```

```
run_kmeans <- coseq(object=countmat, K=2:20, transformation="logclr",
                    model="kmeans")
run_kmeans
```

transformRNAseq *Transform RNA-seq data using common transformations*

Description

Application of common transformations for RNA-seq data prior to fitting a normal mixture model

Usage

```
transformRNAseq(
  y,
  normFactors = "TMM",
  transformation = "arcsin",
  geneLength = NA,
  meanFilterCutoff = NULL,
  verbose = TRUE
)
```

Arguments

y	($n \times q$) matrix or data.frame of observed counts for n observations and q variables
normFactors	The type of estimator to be used to normalize for differences in library size: "TC" for total count, "DESeq" for the normalization method in the DESeq package, and "TMM" for the TMM normalization method (Robinson and Oshlack, 2010). Can also be a vector (of length q) containing pre-estimated library size estimates for each sample.
transformation	Transformation type to be used: "arcsin", "logit", "logMedianRef", "profile", "voom", "logRPKM" (if geneLength is provided by user), "logclr", "clr", "alr", "ilr", "none",
geneLength	Vector of length equal to the number of rows in "y" providing the gene length (bp) for RPKM calculation
meanFilterCutoff	Value used to filter low mean normalized counts
verbose	If TRUE, include verbose output

Value

tcounts	Transformed counts
normCounts	Normalized counts
snorm	Per-sample normalization factors divided by mean normalization factor
ellnorm	Per-sample normalization factors

Examples

```
set.seed(12345)
countmat <- matrix(runif(300*4, min=0, max=500), nrow=300, ncol=4)
countmat <- countmat[which(rowSums(countmat) > 0),]
conds <- rep(c("A","B","C","D"), each=2)

## Arcsin transformation, TMM normalization
arcsin <- transformRNAseq(countmat, normFactors="TMM", transformation="arcsin")$tcounts
## Logit transformation, TMM normalization
logit <- transformRNAseq(countmat, normFactors="TMM", transformation="logit")$tcounts
## logCLR transformation, TMM normalization
logclr <- transformRNAseq(countmat, normFactors="TMM", transformation="logclr")$tcounts
```

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