Package ‘CountClust’

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Type Package
Title Clustering and Visualizing RNA-Seq Expression Data using Grade of Membership Models
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Description Fits grade of membership models (GoM, also known as admixture models) to cluster RNA-seq gene expression count data, identifies characteristic genes driving cluster memberships, and provides a visual summary of the cluster memberships.
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biocViews RNASeq, GeneExpression, Clustering, Sequencing, StatisticalMethod, Software, Visualization
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R topics documented:

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BatchCorrectedCounts

AbundanceGoM

Description

GoM model fit for abundance data

Usage

AbundanceGoM

Format

A list of GoM model output

Value

A list of GoM model output

BatchCorrectedCounts

Obtain Batch effect Corrected counts

Description

This function first converts counts data to log CPM data, then apply a linear model with the batch effect as a factor. We take the sum of intercept, residuals and mean batch effect across all the batches and then inverse transform it back to counts to get rid of batch effects.

Usage

BatchCorrectedCounts(data, batch_lab, use_parallel = TRUE)
cg_topics

**Arguments**

data  count matrix, with samples along the rows and features along the columns.
batch_lab  batch label vector.
use_parallel  if TRUE, we do a parallel analysis over features, else serial application.

**Value**

Returns a counts data. with same dimension as the input data, but which is corrected for batch_lab.

**Examples**

```r
# Simulation example
N=500;
K=4;
G=100;
Label.Batch=c(rep(1,N/4),rep(2,N/4),rep(3,N/4),rep(4,N/4));
alpha_true=matrix(rnorm((K)*G,0.5,1),nrow=(K));
library(gtools)
tt <- 10;
omega_true = matrix(rbind(rdirichlet(tt*10,c(3,4,2,6)),
                           rdirichlet(tt*10,c(1,4,6,3)),
                           rdirichlet(tt*10,c(4,1,2,2)),
                           rdirichlet(tt*10,c(2,6,3,2)),
                           rdirichlet(tt*10,c(3,3,5,4))), nrow=N);
B=max(Label.Batch);
sigmab_true=2;
beta_true=matrix(0,B,G);
for(g in 1:G) {
  beta_true[,g]=rnorm(B,mean=0,sd=sigmab_true);
}
read_counts=matrix(0,N,G);
for(n in 1:N) {
  for(g in 1:G) {
    read_counts[n,g]=rpois(1, omega_true[n,]%*%exp(alpha_true[,g]
                        + beta_true[Label.Batch[n],g]));
  }
}
batchcorrect_counts <- BatchCorrectedCounts(read_counts, Label.Batch,
                                          use_parallel=FALSE)
```

---

**cg_topics**  
Compute the center of gravity of the clusters

**Description**

This function computes the center of gravity for each cluster by taking weighted mean of each component of features where the weights are determined from the theta matrix of the topic model fit.
compare_omega

Usage

cg_topics(theta, feature.comp)

Arguments

theta        The cluster probability distribution/theta matrix obtained from the GoM model fitting (it is a G x K matrix where G is number of features, K number of topics)
feature.comp the numeric matrix (G x L) comprising of values for each feature g and feature metadata l.

Value

Returns a matrix of cluster centers of gravity for the L feature metadata.

Examples

N=360;
M=560;
lat <- rep(1:N, M);
long <- rep(1:M, each=N)
comp <- cbind(lat, long);
data(AbundanceGoM)
center_gravity <- cg_topics(AbundanceGoM$theta, comp);

compare_omega

Re-ordering cluster membership proportion matrices and Information calculation

Description

This function computes a re-ordering of the clusters from GoM model fit in one model to make it comparable with that from another. The two models are applied on the same set of samples with same number of clusters, but the features may change from one model to another. The two models may not be of same type as well. One could be a DAPC model, the other a standard topic model. Aids in checking for consistency in topic proportion patterns across multiple GoM methods or across different types of feature sets.

Usage

compare_omega(omega1, omega2)

Arguments

omega1        cluster membership proportion matrix (N x K) from model 1
omega2        cluster membership proportion matrix (N x K) from model 2
Value

Returns a list containing

kl.dist A symmetric KL divergence matrix across the re-ordered clusters of two omega matrices

kl.order_model2_topics re-ordering of the clusters for omega2 to match the clusters for omega1 based on KL divergence

kl.information_content A measure based on KL information to record how much information in omega2 is explained by omega1. Varies from 0 to 1

cor.dist A correlation matrix across the re-ordered clusters of two omega matrices

cor.order_model2_topics re-ordering of the clusters for omega2 to match the clusters for omega1 based on correlation information

cor.information_content A measure based on correlation information to record how much information in omega2 is explained by omega1. Varies from 0 to 1

Examples

```r
tt=10;
omega1=matrix(rbind(gtools::rdirichlet(tt*10,c(3,4,2,6)),
gtools::rdirichlet(tt*10,c(1,4,6,3)),
gtools::rdirichlet(tt*10,c(4,1,2,2))), nrow=3*10*tt);
omega2=matrix(rbind(gtools::rdirichlet(tt*10,c(1,2,4,6)),
gtools::rdirichlet(tt*10,c(1,4,6,3)),
gtools::rdirichlet(tt*10,c(3,1,5,2))), nrow=3*10*tt);
out <- compare_omega(omega1, omega2)
```

Description

This function takes the FitGoM output model fits and compute log likelihood, BIC and null model loglikelihood for the GoM models.

Usage

```r
compGoM(data, model_output)
```

Arguments

data matrix on which GoM model is fitted (samples along rows, genes along columns)

model_output FitGoM output (a list).
compGoM_models a vector of GoM model fit BIC, loglikelihood and null model loglikelihood for each model in FiGoM model input.

Examples

```r
read.data <- function() {
  x <- tempfile()
  download.file(paste0("https://cdn.rawgit.com/kkdey/",
                        "singleCellRNASeqMouseDeng2014",
                        "/master/data/Deng2014MouseEsc.rda"),
                        destfile = x, quiet = TRUE)
  z <- get(load(x))
  return(z)
}
Deng2014MouseESC <- read.data()

# Extract observed counts
deng.counts <- Biobase::exprs(Deng2014MouseESC)

# Import GoM fitting results
data("MouseDeng2014.FitGoM")
names(MouseDeng2014.FitGoM)

compGoM(data = t(deng.counts),
        model_output = MouseDeng2014.FitGoM)
```

<table>
<thead>
<tr>
<th>ex.counts</th>
<th>counts data for GTEx V6 Brain data for 200 genes</th>
</tr>
</thead>
</table>

Description

counts data for GTEx V6 Brain data for 200 genes

Usage

ex.counts

Format

A data frame 1259 by 200 in dimensions

Value

A data frame 1259 by 200 in dimensions
ExtractHighCorFeatures

Extracting most highly correlated genes with GoM topics/clusters

Description
This function compares grades of membership profile for each cluster in GoM model fit with the
data expression profile to identify genes that are mostly strongly associated with each topic.

Usage
ExtractHighCorFeatures(omega, data, num_genes = 100)

Arguments
omega
- **omega** matrix, the relative grades of memberships from the GoM model fitting
(a \(N \times K\) matrix where \(N\) is number of samples, \(K\) number of topics).
data
- **data** \(G \times N\) matrix of the expression profile of genes across samples, where \(G\) is
the number of features and \(N\) number of samples
num_genes
- The number of top associated genes with each cluster. Defaults to 100

Value
A list containing two items - a \(K \times num\_genes\) matrix of the top strongly associated/correlated
indices/features for \(K\) clusters, and another \(K \times num\_genes\) matrix of the absolute values of the corre-
lations.

Examples
```r
data("MouseDeng2014.FitGoM")
omega_mat <- MouseDeng2014.FitGoM$clust_6$omega;
read.data1 = function() {
  x = tempfile()
  z = get(load((x)))
  return(z)
}
Deng2014MouseESC <- read.data1()
deng.counts <- Biobase::exprs(Deng2014MouseESC)
out <- ExtractHighCorFeatures(omega_mat, deng.counts, num_genes=10)
```

ExtractTopFeatures

Extracting top driving genes driving GoM clusters

Description
This function uses relative gene expression profile of the GoM clusters and applies a KL-divergence
based method to obtain a list of top features that drive each of the clusters.
Usage

ExtractTopFeatures(theta, top_features = 10, method = c("poisson", "bernoulli"), options = c("min", "max"))

Arguments

theta matrix, the relative gene expression profile of the GoM clusters (cluster probability distributions) from the GoM model fitting (a $G \times K$ matrix where $G$ is number of features, $K$ number of topics).

top_features The top features in each cluster $k$ that are selected based on the feature’s ability to distinguish cluster $k$ from cluster $1, \ldots, K$ for all cluster $k \neq l$. Default: 10.

method The underlying model assumed for KL divergence measurement. Two choices considered are "bernoulli" and "poisson". Default: poisson.

options if "min", for each cluster $k$, we select features that maximize the minimum KL divergence of cluster $k$ against all other clusters for each feature. If "max", we select features that maximize the maximum KL divergence of cluster $k$ against all other clusters for each feature.

Value

A matrix (K x top_features) which tabulates in $k$-th row the top feature indices driving the cluster $k$.

Examples

data("MouseDeng2014.FitGoM")
theta_mat <- MouseDeng2014.FitGoM$clust_6$theta;
top_features <- ExtractTopFeatures(theta_mat, top_features=100,
method="poisson", options="min");

FitGoM

Grade of Membership (GoM) model fit!

Description

Fits a grade of membership model to count data. Default input includes a sample-by-feature matrix, the number of clusters (topics) to fit (K). The function is a wrapper of the topics() function implemented in Matt Taddy’s maptpx package.

Usage

FitGoM(data, K, tol = 0.1, path_rda = NULL, control = list())

Arguments

data counts data $N \times G$, with $N$, the number of samples along the rows and $G$, number of genes along columns.

K the vector of clusters or topics to be fitted.
GTExV6Brain.FitGoM

### Description

GoM model fit for GTEx V6 Brain bulk-RNA data

### Usage

```r
GTExV6Brain.FitGoM
```

### Format

A list of GoM model output for k=7

### Value

A list of GoM model output for k=7

---

#### tol

Tolerance value for GoM model absolute log posterior increase at successive iterations (set to 0.1 as default).

#### path_rda

The directory path for saving the GoM model output. If NULL, it will return the output to console.

#### control

Control parameters. Same as topics() function of maptpx package.

### References


### Examples

```r
data("ex.counts")
out <- FitGoM(ex.counts, K=4, tol=100, control=list(tmax=100))
```
handleNA

Deal with NAs in the dataset!

Description

This function handles the NA values in the count data. If for a feature, the proportion of NAs is greater than threshold proportion, then we remove the feature, otherwise we use MAR substitution scheme using the distribution of the non NA values for the feature. If threshold proportion is 0, it implies removal of all features with NA values. Default value of threshold proportion is 0.

Usage

handleNA(data, thresh_prop = 0)

Arguments

data count data in a sample by feature matrix.
thresh_prop threshold proportion of NAs for removal of feature or replacing the NA values.

Details

This function removes NAs from the counts data

Value

Returns a list with

data The modified data with NA substitution and removal
na_removed_cols The columns in the data with NAs that were removed
na_sub_cols The columns in the data with NAs that were substituted

Examples

mat <- rbind(c(2,4,NA),c(4,7,8),c(3,NA,NA));
handleNA(mat,thresh_prop=0.5)
handleNA(mat)

MouseDeng2014.FitGoM

GoM model fit for Deng et al 2014 single cell RNA-seq data on mouse

Description

GoM model fit for Deng et al 2014 single cell RNA-seq data on mouse

Usage

MouseDeng2014.FitGoM
Format

A list of GoM model output for 6 clusters (k=2:7)

Value

A list of GoM model output for 6 clusters (k=2:7)

nullmodel_GoM

Null models for Grade of Membership (GoM) cluster validation

Description

Use null models (popular in ecology) to generate randomized matrix of counts given the observed data matrix, fit the GoM model to these null matrices and compare the fit on null model data with that on the observed data. Used for validating the GoM clusters

Usage

nullmodel_GoM(counts, K, tol = 0.1, null.model = c("frequency", "richness", "independentswap", "trialswap"), iter_fill = 1000, iter_randomized = 100, plot = TRUE)
RemoveSparseFeatures

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>counts</td>
<td>The counts matrix (N x G): N- the number of samples, G- number of features</td>
</tr>
<tr>
<td>K</td>
<td>The number of clusters to fit</td>
</tr>
<tr>
<td>tol</td>
<td>The tolerance of the GoM model fitted</td>
</tr>
<tr>
<td>null.model</td>
<td>The type of nullmodel used (similar to the randomizeMatrix() function argument in picante package)</td>
</tr>
<tr>
<td>iter_fill</td>
<td>The number of swaps/fills in each randomized matrix build</td>
</tr>
<tr>
<td>iter_randomized</td>
<td>The number of randomization matrices generated</td>
</tr>
<tr>
<td>plot</td>
<td>If TRUE, plots density of log Bayes factor</td>
</tr>
</tbody>
</table>

Value

Returns a list with:

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GoMBF.obs</td>
<td>log BF for the observed counts with K=2 against the null with no clusters</td>
</tr>
<tr>
<td>GoMBF.rand</td>
<td>a vector of log BF for each randomized count matrix with K=2 against the null with no clusters</td>
</tr>
<tr>
<td>pval</td>
<td>the p-value of the observed log Bayes factor against the ones from randomized matrices</td>
</tr>
</tbody>
</table>

Examples

```r
data("ex.counts")
nullmodel_GoM(ex.counts,
  K=2,
  tol=500,
  null.model="frequency",
  iter_randomized=3,
  plot=FALSE)
```

RemoveSparseFeatures  Removes features with a lot of 0 counts

Description

This function deals with zero counts in the counts dataset. If for a feature, the proportion of zeros across the samples is greater than filter_prop, then we remove the feature.

Usage

```r
RemoveSparseFeatures(data, filter_prop = 0.9)
```

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>data</td>
<td>count data in a sample by feature matrix.</td>
</tr>
<tr>
<td>filter_prop</td>
<td>threshold proportion. If the proportion of zeros for the feature exceeds this threshold then we remove the feature altogether. Default is 0.9.</td>
</tr>
</tbody>
</table>
StructureGGplot

Value
Returns a list with

- `data`: filtered data with sparse features removed
- `sparse_features`: the feature names of the features found sparse and removed

Examples
```
mat <- rbind(c(2,0,3,0,4),c(4,5,5,0,0),c(30,34,63,25,0),c(0,0,0,0,0));
RemoveSparseFeatures(mat, filter_prop = 0.5)
RemoveSparseFeatures(mat)
```

StructureGGplot

**Struture plot using ggplot2**

Description
Make the traditional Structure plot of GoM model with ggplot2

Usage
```
StructureGGplot(omega, annotation = NULL, palette = RColorBrewer::brewer.pal(8, "Accent"), figure_title = "", yaxis_label = "Tissue type", order_sample = TRUE, sample_order_decreasing = TRUE, split_line = list(split_lwd = 1, split_col = "white"), plot_labels = TRUE, axis_tick = list(axis_ticks_length = 0.1, axis_ticks_lwd_y = 0.1, axis_ticks_lwd_x = 0.1, axis_label_size = 3, axis_label_face = "bold"))
```

Arguments
- **omega**: Cluster membership probabilities of each sample. Usually a sample by cluster matrix in the Topic model output. The cluster weights sum to 1 for each sample.
- **annotation**: data.frame of two columns: sample_id and tissue_label. sample_id is a vetor consisting of character type of variable, which indicates the unique identifying number of each sample. tissue_label is a vector consisting of factor type of variable, which indicates the sample phenotype that is to be used in sorting and grouping the samples in the Structre plot; for example, tissue of origin in making Structure plot of the GTEx samples. Default is set to NULL for when no phenotype information is used to order the sample vectors.
- **palette**: Colors assigned to label the clusters. The first color in the palette is assigned to the cluster that is labeled 1 (usually arbitrarily assigned during the clustering process). Note: The number of colors must be the same or greater than the number of clusters. When the number of clusters is greater than the number of colors, the clusters that are not assigned a color are filled with white in the figure. The recommended choice of color palette is RColorBrewer, for instance RColorBrewer::brewer.pal(8, "Accent") or RColorBrewer::brewer.pal(9, "Set1").
- **figure_title**: Title of the plot.
yaxis_label | Axis label for the phenotype used to order the samples, for example, tissue type or cell type.

order_sample | Whether to order the samples that are of the same tissue label or phenotype label, that is, having the same label in the tissue_label variable. If TRUE, we order samples that are of the same phenotype label and sort the samples by membership of most representative cluster. If FALSE, we keep the order in the data.

sample_order_decreasing | If order_sample=TRUE, then order the sample in descending (TRUE) or ascending order.

split_line | Control parameters for the line that separates phenotype subgroups in the plot.

plot_labels | If TRUE, plots the axis labels.

axis_tick | Control parameters for x-axis and y-axis tick sizes.

**Value**

Plots the Structure plot visualization of the GoM model

**Examples**

```r
# Example 1
data("MouseDeng2014.FitGoM")

# extract the omega matrix: membership weights of each cell
names(MouseDeng2014.FitGoM$clust_6)
omega <- MouseDeng2014.FitGoM$clust_6$omega

# make annotation matrix
annotation <- data.frame(sample_id = paste0("X", c(1:NROW(omega)))
, tissue_label = factor(rownames(omega),
  levels = rev(c("zy", "early2cell",
  "mid2cell", "late2cell",
  "4cell", "8cell", "16cell",
  "earlyblast","midblast",
  "lateblast")))

rownames(omega) <- annotation$sample_id;
StructureGGplot(omega = omega,
  annotation = annotation,
  palette = RColorBrewer::brewer.pal(8, "Accent"),
  yaxis_label = "development phase",
  order_sample = TRUE,
  axis_tick = list(axis_ticks_length = .1,
  axis_ticks_lwd_y = .1,
  axis_ticks_lwd_x = .1,
  axis_label_size = 7,
  axis_label_face = "bold")

# Example 2
# Import Deng et al data

# function to read Deng data from GitHub
read.data <- function() {
  x <- tempfile()
  ```
```
download.file(paste0("https://cdn.rawgit.com/kkdey/",
    "singleCellRNASeqMouseDeng2014",
    "/master/data/Deng2014MouseEsc.rda"),
    destfile = x, quiet = TRUE)
z <- get(load((x)))
return(z)
}
Deng2014MouseESC <- read.data()
deng.counts <- Biobase::exprs(Deng2014MouseESC)
deng.meta_data <- Biobase::pData(Deng2014MouseESC)
deng.gene_names <- rownames(deng.counts)
samples_subvector <- which(!duplicated(deng.meta_data$cell_type))[1:3]

# Fit GoM on 3 samples with K = 3
fit_k3 <- FitGoM( t(deng.counts[, samples_subvector]),
    K = 3, tol=0.1)

names(fit_k3$clust_3)
omega <- fit_k3$clust_3$omega

# make annotation matrix
annotation <- data.frame(
    sample_id = paste0("X", c(1:NROW(omega))),
    tissue_label = factor( as.character(deng.meta_data$cell_type[samples_subvector]),
        levels = rev( as.character(deng.meta_data$cell_type[samples_subvector]) ) )
)
rownames(omega) <- annotation$sample_id
StructureGGplot(omega = omega,
    annotation = annotation,
    palette = RColorBrewer::brewer.pal(3, "Accent"),
    yaxis_label = "development phase",
    order_sample = TRUE,
    axis_tick = list(axis_ticks_length = .1,
        axis_ticks_lwd_y = .1,
        axis_ticks_lwd_x = .1,
        axis_label_size = 7,
        axis_label_face = "bold")
```
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