Package 'decontX'

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Title Decontamination of single cell genomics data

Version 1.5.1

Description This package contains implementation of DecontX (Yang et al. 2020), a decontamination algorithm for single-cell RNA-seq, and DecontPro (Yin et al. 2023), a decontamination algorithm for single cell protein expression data. DecontX is a novel Bayesian method to computationally estimate and remove RNA contamination in individual cells without empty droplet information. DecontPro is a Bayesian method that estimates the level of contamination from ambient and background sources in CITE-seq ADT dataset and decontaminate the dataset.

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Description

DecontX is a decontamination algorithm for single-cell RNA-seq data. DecontPro is a decontamination and background removal algorithm for single cell protein expression data such as CITE-seq or Total-seq.

References

Stan Development Team (2022). RStan: the R interface to Stan. R package version 2.21.7. https://mc-stan.org

.call_stan_vb

.call_stan_vb

Call Stan variational bayes for inference

Description

Call Stan variational bayes for inference

Usage

```
.call_stan_vb(data, initial_condition)
```

Arguments

 $\begin{tabular}{ll} \mbox{data} & A \mbox{ list of input data for Stan.} \\ \mbox{initial_condition} \end{tabular}$

Initial values for Stan params.

Value

Stan output

Description

Process Stan output.

Usage

```
.process_stan_vb_out(stan_vb_output, dat)
```

Arguments

```
stan_vb_output Stan variational bayes output dat List of data input to stan vb
```

Value

Decomposed counts based on Stan estimate.

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decontPro

Decontaminate using decontPro

Description

Decontaminate using decontPro

Usage

```
decontPro(filtered_counts, cell_type, ...)
## S4 method for signature 'SingleCellExperiment'
decontPro(
  filtered_counts,
 cell_type,
 delta\_sd = 2e-05,
 background_sd = 2e-06,
  ambient_counts = NULL,
)
## S4 method for signature 'Seurat'
decontPro(
  filtered_counts,
  cell_type,
  delta\_sd = 2e-05,
 background_sd = 2e-06,
  ambient_counts = NULL,
)
## S4 method for signature 'ANY'
decontPro(
  filtered_counts,
 cell_type,
 delta_sd = 2e-05,
 background_sd = 2e-06,
  ambient_counts = NULL,
)
```

Arguments

filtered_counts

Count matrix NxM (feature x droplet) with only filtered droplets after cell calling. If this is a SingleCellExperiment or a Seurat object, expect counts in the assay slot.

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Value

A list containing decontaminated counts, and estimated parameters.

Examples

decontX

Contamination estimation with decontX

Description

Identifies contamination from factors such as ambient RNA in single cell genomic datasets.

Usage

```
decontX(x, ...)
## S4 method for signature 'SingleCellExperiment'
decontX(
```

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```
х,
 assayName = "counts",
  z = NULL,
  batch = NULL,
  background = NULL,
 bgAssayName = NULL,
 bgBatch = NULL,
 maxIter = 500,
 delta = c(10, 10),
  estimateDelta = TRUE,
  convergence = 0.001,
  iterLogLik = 10,
  varGenes = 5000,
  dbscanEps = 1,
  seed = 12345,
  logfile = NULL,
  verbose = TRUE
)
## S4 method for signature 'ANY'
decontX(
 Х,
 z = NULL
 batch = NULL,
 background = NULL,
 bgBatch = NULL,
 maxIter = 500,
 delta = c(10, 10),
  estimateDelta = TRUE,
  convergence = 0.001,
  iterLogLik = 10,
  varGenes = 5000,
  dbscanEps = 1,
  seed = 12345,
  logfile = NULL,
  verbose = TRUE
)
```

Arguments

Х

A numeric matrix of counts or a SingleCellExperiment with the matrix located in the assay slot under assayName. Cells in each batch will be subsetted and converted to a sparse matrix of class dgCMatrix from package Matrix before analysis. This object should only contain filtered cells after cell calling. Empty cell barcodes (low expression droplets before cell calling) are not needed to run DecontX.

... For the generic, further arguments to pass to each method.

assayName

Character. Name of the assay to use if x is a SingleCellExperiment.

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Numeric or character vector. Cell cluster labels. If NULL, PCA will be used to reduce the dimensionality of the dataset initially, 'umap' from the 'uwot' package will be used to further reduce the dataset to 2 dimensions and the 'dbscan' function from the 'dbscan' package will be used to identify clusters of broad cell

types. Default NULL.

batch Numeric or character vector. Batch labels for cells. If batch labels are sup-

plied, DecontX is run on cells from each batch separately. Cells run in different channels or assays should be considered different batches. Default NULL.

background A numeric matrix of counts or a SingleCellExperiment with the matrix located

in the assay slot under assayName. It should have the same data format as x except it contains the empty droplets instead of cells. When supplied, empirical distribution of transcripts from these empty droplets will be used as the contam-

ination distribution. Default NULL.

bgAssayName Character. Name of the assay to use if background is a SingleCellExperiment.

Default to same as assayName.

bgBatch Numeric or character vector. Batch labels for background. Its unique values

should be the same as those in batch, such that each batch of cells have their corresponding batch of empty droplets as background, pointed by this parame-

ter. Default to NULL.

maxIter Integer. Maximum iterations of the EM algorithm. Default 500.

delta Numeric Vector of length 2. Concentration parameters for the Dirichlet prior

for the contamination in each cell. The first element is the prior for the native counts while the second element is the prior for the contamination counts. These essentially act as pseudocounts for the native and contamination in each cell. If estimateDelta = TRUE, this is only used to produce a random sample of proportions for an initial value of contamination in each cell. Then fit_dirichlet is used to update delta in each iteration. If estimateDelta = FALSE, then delta is fixed with these values for the entire inference procedure. Fixing delta and setting a high number in the second element will force decontX to be more aggressive and estimate higher levels of contamination at the expense of potentially

removing native expression. Default c(10, 10).

estimateDelta Boolean. Whether to update delta at each iteration.

convergence Numeric. The EM algorithm will be stopped if the maximum difference in the

contamination estimates between the previous and current iterations is less than

this. Default 0.001.

iterLogLik Integer. Calculate log likelihood every iterLogLik iteration. Default 10.

varGenes Integer. The number of variable genes to use in dimensionality reduction be-

fore clustering. Variability is calcualted using modelGeneVar function from the

'scran' package. Used only when z is not provided. Default 5000.

dbscanEps Numeric. The clustering resolution parameter used in 'dbscan' to estimate broad

cell clusters. Used only when z is not provided. Default 1.

seed Integer. Passed to with_seed. For reproducibility, a default value of 12345 is

used. If NULL, no calls to with_seed are made.

logfile Character. Messages will be redirected to a file named logfile. If NULL,

messages will be printed to stdout. Default NULL.

verbose Logical. Whether to print log messages. Default TRUE.

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Value

If x is a matrix-like object, a list will be returned with the following items:

decontXcounts: The decontaminated matrix. Values obtained from the variational inference procedure may be non-integer. However, integer counts can be obtained by rounding, e.g. round(decontXcounts).

contamination: Percentage of contamination in each cell.

estimates: List of estimated parameters for each batch. If z was not supplied, then the UMAP coordinates used to generated cell cluster labels will also be stored here.

z: Cell population/cluster labels used for analysis.

runParams: List of arguments used in the function call.

If x is a SingleCellExperiment, then the decontaminated counts will be stored as an assay and can be accessed with decontXcounts(x). The contamination values and cluster labels will be stored in colData(x). estimates and runParams will be stored in metadata(x) decontX. The UMAPs used to generated cell cluster labels will be stored in reducedDims slot in x.

Author(s)

Shiyi Yang, Yuan Yin, Joshua Campbell

```
# Generate matrix with contamination
s <- simulateContamination(seed = 12345)</pre>
library(SingleCellExperiment)
library(celda)
sce <- SingleCellExperiment(list(counts = s$observedCounts))</pre>
sce <- decontX(sce)</pre>
# Plot contamination on UMAP
plotDecontXContamination(sce)
# Plot decontX cluster labels
umap <- reducedDim(sce)</pre>
celda::plotDimReduceCluster(x = sce$decontX_clusters,
    dim1 = umap[, 1], dim2 = umap[, 2], )
# Plot percentage of marker genes detected
# in each cell cluster before decontamination
s$markers
plotDecontXMarkerPercentage(sce, markers = s$markers, assayName = "counts")
# Plot percentage of marker genes detected
# in each cell cluster after contamination
plotDecontXMarkerPercentage(sce, markers = s$markers,
                             assayName = "decontXcounts")
# Plot percentage of marker genes detected in each cell
# comparing original and decontaminated counts side-by-side
```

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decontXcounts

Get or set decontaminated counts matrix

Description

Gets or sets the decontaminated counts matrix from a a SingleCellExperiment object.

Usage

```
decontXcounts(object, ...)
decontXcounts(object, ...) <- value

## S4 method for signature 'SingleCellExperiment'
decontXcounts(object, ...)

## S4 replacement method for signature 'SingleCellExperiment'
decontXcounts(object, ...) <- value</pre>
```

Arguments

object A SingleCellExperiment object.

... For the generic, further arguments to pass to each method.

value A matrix to save as an assay called decontXcounts

Value

If getting, the assay from object with the name decontXcounts will be returned. If setting, a SingleCellExperiment object will be returned with decontXcounts listed in the assay slot.

See Also

```
assay and assay<-
```

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fastNormProp

Fast normalization for numeric matrix

Description

Fast normalization for numeric matrix

Usage

```
fastNormProp(R_counts, R_alpha)
```

Arguments

R_counts An integer matrix

R_alpha A double value to be added to the matrix as a pseudocount

Value

A numeric matrix where the columns have been normalized to proportions

fastNormPropLog

Fast normalization for numeric matrix

Description

Fast normalization for numeric matrix

Usage

```
fastNormPropLog(R_counts, R_alpha)
```

Arguments

R_counts An integer matrix

R_alpha A double value to be added to the matrix as a pseudocount

Value

A numeric matrix where the columns have been normalized to proportions

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fastNormPropSqrt

Fast normalization for numeric matrix

Description

Fast normalization for numeric matrix

Usage

```
fastNormPropSqrt(R_counts, R_alpha)
```

Arguments

R_counts An integer matrix

R_alpha A double value to be added to the matrix as a pseudocount

Value

A numeric matrix where the columns have been normalized to proportions

nonzero

get row and column indices of none zero elements in the matrix

Description

get row and column indices of none zero elements in the matrix

Usage

```
nonzero(R_counts)
```

Arguments

R_counts

A matrix

Value

An integer matrix where each row is a row, column indices pair

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plotBoxByCluster

Boxplot of features grouped by cell type

Description

Boxplot of features grouped by cell type

Usage

```
plotBoxByCluster(
  counts,
  decontaminated_counts,
  cell_type,
  features,
  file = NULL
)
```

Arguments

file file name to save plot into a pdf. If omit, return ggplot object.

Value

Return a pdf file named file or a ggplot object.

plotDecontXContamination

Plots contamination on UMAP coordinates

Description

A scatter plot of the UMAP dimensions generated by DecontX with cells colored by the estimated percentation of contamation.

Usage

```
plotDecontXContamination(
    x,
    batch = NULL,
    colorScale = c("blue", "green", "yellow", "orange", "red"),
    size = 1
)
```

Arguments

х	Either a SingleCellExperiment with decontX results stored in metadata(x)\$decontX or the result from running decontX on a count matrix.
batch	Character. Batch of cells to plot. If NULL, then the first batch in the list will be selected. Default NULL.
colorScale	Character vector. Contains the color spectrum to be passed to scale_colour_gradientn from package 'ggplot2'. Default c("blue", "green", "yellow", "orange", "red").
size	Numeric. Size of points in the scatterplot. Default 1.

Value

Returns a ggplot object.

Author(s)

Shiyi Yang, Joshua Campbell

See Also

See decontX for a full example of how to estimate and plot contamination.

Examples

```
# Generate matrix with contamination
s <- simulateContamination(seed = 12345)</pre>
library(SingleCellExperiment)
library(celda)
sce <- SingleCellExperiment(list(counts = s$observedCounts))</pre>
sce <- decontX(sce)</pre>
# Plot contamination on UMAP
plotDecontXContamination(sce)
# Plot decontX cluster labels
umap <- reducedDim(sce)</pre>
celda::plotDimReduceCluster(x = sce$decontX_clusters,
    dim1 = umap[, 1], dim2 = umap[, 2], )
# Plot percentage of marker genes detected
# in each cell cluster before decontamination
s$markers
plotDecontXMarkerPercentage(sce, markers = s$markers, assayName = "counts")
# Plot percentage of marker genes detected
# in each cell cluster after contamination
plotDecontXMarkerPercentage(sce, markers = s$markers,
                             assayName = "decontXcounts")
# Plot percentage of marker genes detected in each cell
# comparing original and decontaminated counts side-by-side
plotDecontXMarkerPercentage(sce, markers = s$markers,
                             assayName = c("counts", "decontXcounts"))
# Plot raw counts of indiviual markers genes before
# and after decontamination
plotDecontXMarkerExpression(sce, unlist(s$markers))
```

plotDecontXMarkerExpression

Plots expression of marker genes before and after decontamination

Description

Generates a violin plot that shows the counts of marker genes in cells across specific clusters or cell types. Can be used to view the expression of marker genes in different cell types before and after decontamination with decontX.

Usage

```
plotDecontXMarkerExpression(
```

```
x,
markers,
groupClusters = NULL,
assayName = c("counts", "decontXcounts"),
z = NULL,
exactMatch = TRUE,
by = "rownames",
log1p = FALSE,
ncol = NULL,
plotDots = FALSE,
dotSize = 0.1
)
```

Arguments

x Either a SingleCellExperiment or a matrix-like object of counts.

markers Character Vector or List. A character vector or list of character vectors with the

names of the marker genes of interest.

groupClusters List. A named list that allows cell clusters labels coded in z to be regrouped and

renamed on the fly. For example, list(Tcells=c(1, 2), Bcells=7) would recode clusters 1 and 2 to "Tcells" and cluster 7 to "Bcells". Note that if this is used, clusters in z not found in groupClusters will be excluded. Default NULL.

assayName Character vector. Name(s) of the assay(s) to plot if x is a SingleCellExperiment.

If more than one assay is listed, then side-by-side violin plots will be generated.

Default c("counts", "decontXcounts").

z Character, Integer, or Vector. Indicates the cluster labels for each cell. If x is a

SingleCellExperiment and z = NULL, then the cluster labels from decontX will be retreived from the colData of x (i.e. colData(x)\$decontX_clusters). If z is a single character or integer, then that column will be retrived from colData of x. (i.e. colData(x)[,z]). If x is a counts matrix, then z will need to be a vector the same length as the number of columns in x that indicate the cluster to

which each cell belongs. Default NULL.

exactMatch Boolean. Whether to only identify exact matches for the markers or to iden-

tify partial matches using grep. See retrieveFeatureIndex for more details.

Default TRUE.

by Character. Where to search for the markers if x is a SingleCellExperiment. See

retrieveFeatureIndex for more details. If x is a matrix, then this must be set

to "rownames". Default "rownames".

log1p Boolean. Whether to apply the function log1p to the data before plotting. This

function will add a pseudocount of 1 and then log transform the expression val-

ues. Default FALSE.

ncol Integer. Number of columns to make in the plot. Default NULL.

plotDots Boolean. If TRUE, the expression of features will be plotted as points in addition

to the violin curve. Default FALSE.

dotSize Numeric. Size of points if plotDots = TRUE. Default 0.1.

Value

Returns a ggplot object.

Author(s)

Shiyi Yang, Joshua Campbell

See Also

See decontX for a full example of how to estimate and plot contamination.

```
# Generate matrix with contamination
s <- simulateContamination(seed = 12345)</pre>
library(SingleCellExperiment)
library(celda)
sce <- SingleCellExperiment(list(counts = s$observedCounts))</pre>
sce <- decontX(sce)</pre>
# Plot contamination on UMAP
plotDecontXContamination(sce)
# Plot decontX cluster labels
umap <- reducedDim(sce)</pre>
celda::plotDimReduceCluster(x = sce$decontX_clusters,
    dim1 = umap[, 1], dim2 = umap[, 2], )
# Plot percentage of marker genes detected
# in each cell cluster before decontamination
plotDecontXMarkerPercentage(sce, markers = s$markers, assayName = "counts")
# Plot percentage of marker genes detected
# in each cell cluster after contamination
plotDecontXMarkerPercentage(sce, markers = s$markers,
                             assayName = "decontXcounts")
# Plot percentage of marker genes detected in each cell
# comparing original and decontaminated counts side-by-side
plotDecontXMarkerPercentage(sce, markers = s$markers,
                            assayName = c("counts", "decontXcounts"))
# Plot raw counts of indiviual markers genes before
# and after decontamination
plotDecontXMarkerExpression(sce, unlist(s$markers))
```

plotDecontXMarkerPercentage

Plots percentage of cells cell types expressing markers

Description

Generates a barplot that shows the percentage of cells within clusters or cell types that have detectable levels of given marker genes. Can be used to view the expression of marker genes in different cell types before and after decontamination with decontX.

Usage

```
plotDecontXMarkerPercentage(
    x,
    markers,
    groupClusters = NULL,
    assayName = c("counts", "decontXcounts"),
    z = NULL,
    threshold = 1,
    exactMatch = TRUE,
    by = "rownames",
    ncol = round(sqrt(length(markers))),
    labelBars = TRUE,
    labelSize = 3
)
```

Arguments

Z

	T14 01 1 0 11T 1	
Y	Hither a Single Cell Experiment	or a matrix-like object of counts.

markers List. A named list indicating the marker genes for each cell type of interest. Mul-

tiple markers can be supplied for each cell type. For example, $list(Tcell_Markers=c("CD3E", and arkers=c("CD3E"))$

"CD3D"), Bcell_Markers=c("CD79A", "CD79B", "MS4A1") would specify markers for human T-cells and B-cells. A cell will be considered "positive" for a cell type if it has a count greater than threshold for at least one of the marker genes

in the list.

groupClusters List. A named list that allows cell clusters labels coded in z to be regrouped and

renamed on the fly. For example, list(Tcells=c(1, 2), Bcells=7) would recode clusters 1 and 2 to "Tcells" and cluster 7 to "Bcells". Note that if this is used, clusters in z not found in groupClusters will be excluded from the

barplot. Default NULL.

assayName Character vector. Name(s) of the assay(s) to plot if x is a SingleCellExperiment.

If more than one assay is listed, then side-by-side barplots will be generated.

 $Default \ \verb|c("counts", "decontXcounts")|.$

Character, Integer, or Vector. Indicates the cluster labels for each cell. If x is a

SingleCellExperiment and z = NULL, then the cluster labels from decontX will be retived from the colData of x (i.e. colData(x)\$decontX_clusters). If z

is a single character or integer, then that column will be retrived from colData of x. (i.e. colData(x)[,z]). If x is a counts matrix, then z will need to be a vector the same length as the number of columns in x that indicate the cluster to

which each cell belongs. Default NULL.

threshold Numeric. Markers greater than or equal to this value will be considered detected

in a cell. Default 1.

Boolean. Whether to only identify exact matches for the markers or to idenexactMatch

tify partial matches using grep. See retrieveFeatureIndex for more details.

Default TRUE.

by Character. Where to search for the markers if x is a SingleCellExperiment. See

retrieveFeatureIndex for more details. If x is a matrix, then this must be set

to "rownames". Default "rownames".

ncol Integer. Number of columns to make in the plot. Default round(sqrt(length(markers)).

labelBars Boolean. Whether to display percentages above each bar Default TRUE.

labelSize Numeric. Size of the percentage labels in the barplot. Default 3.

Value

Returns a ggplot object.

Author(s)

Shiyi Yang, Joshua Campbell

See Also

See decontX for a full example of how to estimate and plot contamination.

```
# Generate matrix with contamination
s <- simulateContamination(seed = 12345)</pre>
library(SingleCellExperiment)
library(celda)
sce <- SingleCellExperiment(list(counts = s$observedCounts))</pre>
sce <- decontX(sce)</pre>
# Plot contamination on UMAP
plotDecontXContamination(sce)
# Plot decontX cluster labels
umap <- reducedDim(sce)</pre>
celda::plotDimReduceCluster(x = sce$decontX_clusters,
    dim1 = umap[, 1], dim2 = umap[, 2], )
# Plot percentage of marker genes detected
# in each cell cluster before decontamination
s$markers
```

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plotDensity

Density of each ADT, raw counts overlapped with decontaminated counts

Description

Density of each ADT, raw counts overlapped with decontaminated counts

Usage

```
plotDensity(counts, decontaminated_counts, features, file = NULL)
```

Arguments

counts original count matrix of nADT x nDroplet.

decontaminated_counts

decontaminated count matrix.

features names of ADT to plot

file file name to save plot into a pdf. If omit, return ggplot object.

Value

Return a pdf file named file or a ggplot object.

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retrieveFeatureIndex Retrieve row index for a set of features

Description

This will return indices of features among the rownames or rowData of a data.frame, matrix, or a SummarizedExperiment object including a SingleCellExperiment. Partial matching (i.e. grepping) can be used by setting exactMatch = FALSE.

Usage

```
retrieveFeatureIndex(
  features,
  x,
  by = "rownames",
  exactMatch = TRUE,
  removeNA = FALSE
)
```

Arguments

features	Character vector of feature names to find in the rows of x.
x	A data.frame, matrix, or SingleCellExperiment object to search.
by	Character. Where to search for features in x. If set to "rownames" then the features will be searched for among rownames(x). If x inherits from class SummarizedExperiment, then by can be one of the fields in the row annotation data.frame (i.e. one of colnames(rowData(x))).
exactMatch	Boolean. Whether to only identify exact matches or to identify partial matches using grep.
removeNA	Boolean. If set to FALSE, features not found in x will be given NA and the returned vector will be the same length as features. If set to TRUE, then the NA values will be removed from the returned vector. Default FALSE.

Value

A vector of row indices for the matching features in x.

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Author(s)

Yusuke Koga, Joshua Campbell

See Also

'retrieveFeatureInfo' from package 'scater' and link{regex} for how to use regular expressions when exactMatch = FALSE.

Examples

simulateContamination Simulate contaminated count matrix

Description

This function generates a list containing two count matrices – one for real expression, the other one for contamination, as well as other parameters used in the simulation which can be useful for running decontamination.

Usage

```
simulateContamination(
   C = 300,
   G = 100,
   K = 3,
   NRange = c(500, 1000),
   beta = 0.1,
   delta = c(1, 10),
   numMarkers = 3,
   seed = 12345
)
```

Arguments

C Integer. Number of cells to be simulated. Default 300.

G Integer. Number of genes to be simulated. Default 100.

K Integer. Number of cell populations to be simulated. Default 3.

NRange Integer vector. A vector of length 2 that specifies the lower and upper bounds of

the number of counts generated for each cell. Default c(500, 1000).

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beta Numeric. Concentration parameter for Phi. Default 0.1.

delta Numeric or Numeric vector. Concentration parameter for Theta. If input as

a single numeric value, symmetric values for beta distribution are specified; if input as a vector of lenght 2, the two values will be the shape1 and shape2

paramters of the beta distribution respectively. Default c(1, 5).

numMarkers Integer. Number of markers for each cell population. Default 3.

seed Integer. Passed to with_seed. For reproducibility, a default value of 12345 is

used. If NULL, no calls to with_seed are made.

Value

A list containing the nativeMatirx (real expression), observedMatrix (real expression + contamination), as well as other parameters used in the simulation.

Author(s)

Shiyi Yang, Yuan Yin, Joshua Campbell

Examples

contaminationSim <- simulateContamination(K = 3, delta = c(1, 10))

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