

Package ‘Canek’

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Type Package

Title Batch Correction of Single Cell Transcriptome Data

Version 0.2.5

Description Non-linear/linear hybrid method for batch-effect correction that uses Mutual Nearest Neighbors (MNNs) to identify similar cells between datasets. Reference: Loza M. et al. (NAR Genomics and Bioinformatics, 2020) <[doi:10.1093/nargab/lqac022](https://doi.org/10.1093/nargab/lqac022)>.

Depends R (>= 3.5.0)

Imports FNN, irlba, numbers, fpc, bluster, igraph, matrixStats, utils

Suggests testthat (>= 2.1.0), Seurat, SingleCellExperiment, SummarizedExperiment, scater, batchelor, scran, knitr, rmarkdown, patchwork, ggplot2

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Encoding UTF-8

LazyData true

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VignetteBuilder knitr

URL <https://martinloza.github.io/Canek/>

BugReports <https://github.com/MartinLoza/Canek/issues>

NeedsCompilation no

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Contents

| | |
|--------------------------|-----------|
| CheckZeroCV | 2 |
| CorrectBatch | 3 |
| CorrectBatches | 5 |
| EkfBE | 7 |
| Fuzzy | 8 |
| MeanBE | 9 |
| MedianBE | 9 |
| PairsFiltering | 10 |
| RunCanek | 11 |
| SimBatches | 12 |
| Index | 13 |

| | |
|-------------|--------------------|
| CheckZeroCV | <i>CheckZeroCV</i> |
|-------------|--------------------|

Description

CheckZeroCV

Usage

```

CheckZeroCV(
    MST = NULL,
    cluMem = NULL,
    corGene = NULL,
    fuzzyPCA = fuzzyPCA,
    memCorrData = NULL,
    zeroCorrection = NULL
)

```

Arguments

| | |
|----------------|---|
| MST | Minimum Spanning Tree |
| cluMem | Clusters used on MST |
| corGene | Data to correct |
| fuzzyPCA | Number of PCs to use in the fuzzy process. |
| memCorrData | Data to correct |
| zeroCorrection | Vector indicating which membership has a zero correction vector |

 CorrectBatch

CorrectBatch

Description

Batch effect correction on two single-cell batches

Usage

```
CorrectBatch(
  refBatch,
  queBatch,
  cnRef = NULL,
  cnQue = NULL,
  queNumCelltypes = NULL,
  maxMem = 5,
  pairs = NULL,
  kNN = 30,
  sampling = FALSE,
  numSamples = NULL,
  idxQuery = NULL,
  idxRef = NULL,
  pcaDim = 50,
  perCellMNN = 0.08,
  fuzzy = TRUE,
  fuzzyPCA = 10,
  estMethod = "Median",
  clusterMethod = "louvain",
  pairsFilter = FALSE,
  doCosNorm = FALSE,
  verbose = FALSE
)
```

Arguments

| | |
|-----------------|---|
| refBatch | Reference batch. |
| queBatch | Query batch (batch to correct). |
| cnRef | Cosine normalization of the reference batch. |
| cnQue | Cosine normalization of the query batch. |
| queNumCelltypes | Number of cell types in the query batch. By default Canek searches the number of cell types using an heuristic algorithm. Change this parameter if you know the number of cell types in advanced. |
| maxMem | Maximum number of memberships from the query batch. This parameter is used on the heuristic algorithm to find the number of cell types. |

| | |
|----------------------------|---|
| <code>pairs</code> | A numerical matrix containing MNNs pairs cell indexes. First column corresponds to query batch cell indexes. |
| <code>kNN</code> | Number of k-nearest-neighbors used to define the MNNs pairs. |
| <code>sampling</code> | Use MNNs pairs sampling when using a Kalman filter to estimate the correction vector. |
| <code>numSamples</code> | If sampling. Number of MNNs pairs samples to use on the estimation process. |
| <code>idxQuery</code> | Numerical vector indicating the index of the cells from the query batch to use on the correction vector estimation. |
| <code>idxRef</code> | Numerical vector indicating the index of the cells from the reference batch to use on the correction vector estimation. |
| <code>pcaDim</code> | Number of PCA dimensions to use. |
| <code>perCellMNN</code> | Threshold value to decide if a membership's correction value is calculated. As a rough interpretation, this values can be thought as the proportion of cells from a membership with an associated MNN pair. If the proportion is low, an specific correction vectors is not calculated for this membership. |
| <code>fuzzy</code> | Use fuzzy logic to join the local correction vectors. |
| <code>fuzzyPCA</code> | Number of PCs to use in the fuzzy process. |
| <code>estMethod</code> | Method to use when estimating the correction vectors: <ul style="list-style-type: none"> • Median. Use the cells median distance. • EKF. Use an extended Kalman filter. |
| <code>clusterMethod</code> | Method used to identify memberships. |
| <code>pairsFilter</code> | Filter MNNs pairs before estimating the correction vectors. If TRUE, the pairs are filtered from outliers using an interquartile range method. |
| <code>doCosNorm</code> | Whether to do cosine normalization. |
| <code>verbose</code> | Print output. |

Details

CorrectBatch is a method to correct batch-effect from two single-cell batches. Batch-effects observations are defined using mutual nearest neighbors (MNNs) pairs and cell groups from the query batch are distinguished using clustering. We estimate a correction vector for each cluster using its MNNs pairs and use these vectors to remove the batch effect from the query batch in two ways:

- A linear correction is performed by equally correcting the cells from the same cluster.
- A non-linear correction is performed by differently correcting each cell using fuzzy logic.

Value

A list containing the input batches, the corrected query batch, and the correction data

Examples

```

x <- SimBatches$batches[[1]]
y <- SimBatches$batches[[2]]
z <- CorrectBatch(x, y)
Corrected <- z$`Corrected Query Batch`

Uncorrected_PCA <- prcomp(t(cbind(x,y)))
plot(Uncorrected_PCA$x[,1:2])
Corrected_PCA <- prcomp(t(cbind(x,z$`Corrected Query Batch`)))
plot(Corrected_PCA$x[,1:2])

```

| | |
|----------------|-----------------------|
| CorrectBatches | <i>CorrectBatches</i> |
|----------------|-----------------------|

Description

Batch-effect correction over a list of single cell batches

Usage

```

CorrectBatches(
  lsBatches,
  hierarchical = TRUE,
  queNumCelltypes = NULL,
  maxMem = 5,
  sampling = FALSE,
  numSamples = NULL,
  kNN = 30,
  pcaDim = 50,
  pairsFilter = FALSE,
  perCellMNN = 0.08,
  fuzzy = TRUE,
  fuzzyPCA = 10,
  estMethod = "Median",
  clusterMethod = "louvain",
  doCosNorm = FALSE,
  fracSampling = NULL,
  debug = FALSE,
  verbose = FALSE,
  ...
)

```

Arguments

lsBatches List of batches to integrate. Batches should contain the same number of genes as rows.

| | |
|-----------------|---|
| hierarchical | Use hierarchical integration scheme when correcting more than two batches. If set to FALSE, the input batches are sorted by number of cells and integrated on descending order. |
| queNumCelltypes | Number of cell types in the query batch. By default Canek searches the number of cell types using an heuristic algorithm. Change this parameter if you know the number of cell types in advanced. |
| maxMem | Maximum number of memberships from the query batch. This parameter is used on the heuristic algorithm to find the number of cell types. |
| sampling | Use MNNs pairs sampling when using a Kalman filter to estimate the correction vector. |
| numSamples | If sampling. Number of MNNs pairs samples to use on the estimation process. |
| kNN | Number of k-nearest-neighbors used to define the MNNs pairs. |
| pcaDim | Number of PCA dimensions to use. |
| pairsFilter | Filter MNNs pairs before estimating the correction vectors. If TRUE, the pairs are filtered from outliers using an interquartile range method. |
| perCellMNN | Threshold value to decide if a membership's correction value is calculated. As a rough interpretation, this values can be thought as the proportion of cells from a membership with an associated MNN pair. If the proportion is low, an specific correction vectors is not calculated for this membership. |
| fuzzy | Use fuzzy logic to join the local correction vectors. |
| fuzzyPCA | Number of PCs to use in the fuzzy process. |
| estMethod | Method to use when estimating the correction vectors: <ul style="list-style-type: none"> • Median. Use the cells median distance • EKF. Use an extended Kalman filter |
| clusterMethod | Method used to identify memberships. |
| doCosNorm | Whether to do cosine normalization. |
| fracSampling | Fraction of cells to sample in the hierarchical selection (default is NULL, no sampling). |
| debug | Return correction's information |
| verbose | Print output. |
| ... | Pass down methods from RunCanek(). |

Details

CorrectBatches is a method to correct batch-effect from two or more single-cell batches. Batch-effects observations are defined using mutual nearest neighbors (MNNs) pairs and cell groups from the query batch are distinguished using clustering. We estimate a correction vector for each cluster using its MNNs pairs and use these vectors to remove the batch effect from the query batch in two ways:

- A linear correction is performed by equally correcting the cells from the same cluster.
- A non-linear correction is performed by differently correcting each cell using fuzzy logic.

Value

A list containing the integrated datasets as matrix and the correction data .

Examples

```
Batches <- SimBatches$batches
z <- CorrectBatches(Batches)

Uncorrected_PCA <- prcomp(t(cbind(Batches[[1]], Batches[[2]])))
plot(Uncorrected_PCA$x[,1:2])
Corrected_PCA <- prcomp(t(z))
plot(Corrected_PCA$x[,1:2])
```

 EkfBE

Correction vector estimation

Description

Batch effect estimation using an extended Kalman filter

Usage

```
EkfBE(
  refBatch,
  queBatch,
  pairs,
  sampling = FALSE,
  numSamples = NULL,
  verbose = FALSE
)
```

Arguments

| | |
|------------|---|
| refBatch | Reference batch. |
| queBatch | Query batch. |
| pairs | A numerical matrix containing MNNs pairs cell indexes. First column corresponds to query batch cells. |
| sampling | Sample MNNs pairs. |
| numSamples | If sampling, number of MNNs pairs samples to use on the estimation process. |
| verbose | Print output. |

Details

The input batches must have the same number of genes. The model used on the estimation has the form of $g_{ref} = g_{que} + be$, where the batch effect is represented as a value added to the reference gene expression, causing a linear deviation between the reference and the query batches.

Value

A list containing the estimated correction vector and the estimation data. The length of the correction vector is equal to the number of genes.

 Fuzzy

Title Fuzzy

Description

Function to score cell's memberships by fuzzy logic

Usage

```
Fuzzy(
  cluMem = NULL,
  pcaQue = NULL,
  corCell = NULL,
  fuzzyPCA = 10,
  MST = NULL,
  verbose = FALSE
)
```

Arguments

| | |
|----------|--|
| cluMem | Memberships' clustering data. |
| pcaQue | PCA representation of the cells. |
| corCell | Matrix containing the initial membership assignment. Matrix dimensions are expected as #Cell x #Memberships, with each row sum equal to 1. |
| fuzzyPCA | Number of PCs to use in the fuzzy process. |
| MST | Minimum spanning tree |
| verbose | Print output. |

Details

This function perform the fuzzification for the cells' membership. A minimum spanning tree (MST) is created among memberships, and the fuzzification is performed for each of the edges of the MST.#'

| | |
|--------|---------------|
| MeanBE | <i>MeanBE</i> |
|--------|---------------|

Description

Batch effect estimation using the MNNs pairs.

Usage

```
MeanBE(refBatch, queBatch, pairs)
```

Arguments

| | |
|----------|---|
| refBatch | Reference batch. |
| queBatch | Query batch. |
| pairs | A numerical matrix containing MNNs pairs cell indexes. First column corresponds to query batch cells. |

Details

The input batches must have the same number of genes. The model used on the estimation has the form of $g_ref = g_que + be$, where the batch effect is represented as a value added to the reference gene expression. The batch effect is estimated as the median of the gene expression difference among the reference and the query batch, e.g. $Median(g_ref - g_que)$.

Value

A list containing the estimated correction vector and the estimation data. The length of the correction vector is equal to the number of genes.

| | |
|----------|-------------------------------------|
| MedianBE | <i>Correction vector estimation</i> |
|----------|-------------------------------------|

Description

Batch effect estimation using the MNNs pairs.

Usage

```
MedianBE(refBatch, queBatch, pairs)
```

Arguments

| | |
|----------|---|
| refBatch | Reference batch. |
| queBatch | Query batch. |
| pairs | A numerical matrix containing MNNs pairs cell indexes. First column corresponds to query batch cells. |

Details

The input batches must have the same number of genes. The model used on the estimation has the form of $g_ref = g_que + be$, where the batch effect is represented as a value added to the reference gene expression. The batch effect is estimated as the median of the gene expression difference among the reference and the query batch, e.g. $Median(g_ref - g_que)$.

Value

A list containing the estimated correction vector and the estimation data. The length of the correction vector is equal to the number of genes.

| | |
|----------------|-----------------------------|
| PairsFiltering | <i>Title PairsFiltering</i> |
|----------------|-----------------------------|

Description

Function to filter MNNs pairs

Usage

```
PairsFiltering(refBatch, queBatch, pairs, verbose = FALSE)
```

Arguments

| | |
|----------|---|
| refBatch | Reference batch single-cell data. |
| queBatch | Query's batch single-cell data. |
| pairs | A matrix containing MNNs pairs. First column corresponds to query-batch cell indexes. |
| verbose | Print output. |

Details

Filter MNN pairs by quantiles.

Value

A matrix containing the filtered pairs. First column corresponds to query-batch cell indexes.

RunCanek

*RunCanek***Description**

Runs Canek integration.

Usage

```
RunCanek(x, ...)

## S3 method for class 'Seurat'
RunCanek(
  x,
  batches = NULL,
  slot = "data",
  assay = NULL,
  features = NULL,
  selection.method = "vst",
  nfeatures = 2000,
  fvf.nfeatures = 2000,
  integration.name = "Canek",
  debug = FALSE,
  ...
)

## S3 method for class 'SingleCellExperiment'
RunCanek(
  x,
  batches = NULL,
  assay = "logcounts",
  integration.name = "Canek",
  debug = FALSE,
  ...
)

## S3 method for class 'list'
RunCanek(x, ...)
```

Arguments

| | |
|----------------------|---|
| <code>x</code> | object with expression counts or list of matrices. |
| <code>...</code> | additional arguments passed down to methods. |
| <code>batches</code> | for S4 objects the column containing batch information. |
| <code>slot</code> | slot used for Seurat objects (default: data). |
| <code>assay</code> | assay used for Seurat objects. |

features optional vector of features to use for correction.
selection.method method used for FindVariableFeatures on Seurat objects when features is NULL.
nfeatures number of features returned by SelectIntegrationFeatures.
fvf.nfeatures number of features returned by FindVariableFeatures.
integration.name name for the integrated assay.
debug whether to store information about correction vector.

Value

An object of the appropriate type.

| | |
|------------|---|
| SimBatches | <i>Dataset with simulated single cell RNA-seq from 2 batches.</i> |
|------------|---|

Description

Dataset with simulated single cell RNA-seq from 2 batches.

Usage

```
SimBatches
```

Format

A list with the following elements:

batches a list with two matrices representing the two batches

pairs matrix of pairs between the two batches.

cell_types a factor with the cell clusters. ...

Index

* datasets

- SimBatches, [12](#)

- CheckZeroCV, [2](#)
- CorrectBatch, [3](#)
- CorrectBatches, [5](#)

- EkfBE, [7](#)

- Fuzzy, [8](#)

- MeanBE, [9](#)
- MedianBE, [9](#)

- PairsFiltering, [10](#)

- RunCaneK, [11](#)

- SimBatches, [12](#)