

# Package ‘scMerge’

October 17, 2020

**Type** Package

**Title** scMerge: Merging multiple batches of scRNA-seq data

**Version** 1.4.0

**Description** Like all gene expression data, single-cell RNA-seq (scRNA-Seq) data suffers from batch effects and other unwanted variations that makes accurate biological interpretations difficult. The scMerge method leverages factor analysis, stably expressed genes (SEGs) and (pseudo-) replicates to remove unwanted variations and merge multiple scRNA-Seq data. This package contains all the necessary functions in the scMerge pipeline, including the identification of SEGs, replication-identification methods, and merging of scRNA-Seq data.

**License** GPL-3

**Encoding** UTF-8

**LazyData** false

**Depends** R (>= 3.6.0)

**Imports** BiocParallel, BiocSingular, cluster, DelayedArray, DelayedMatrixStats, distr, dplyr, igraph, M3Drop (>= 1.9.4), parallel, pdist, proxy, Rcpp (>= 0.12.18), RcppEigen (>= 0.3.3.4.0), ruv, S4Vectors (>= 0.23.19), SingleCellExperiment (>= 1.7.3), SummarizedExperiment

**LinkingTo** Rcpp (>= 0.12.18), RcppEigen, testthat

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**VignetteBuilder** knitr

**biocViews** BatchEffect, GeneExpression, Normalization, RNASeq, Sequencing, SingleCell, Software, Transcriptomics

**URL** <https://github.com/SydneyBioX/scMerge>

**BugReports** <https://github.com/SydneyBioX/scMerge/issues>

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eigenMatMult	<i>Fast matrix multiplication using RcppEigen</i>
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### Description

Fast matrix multiplication using RcppEigen

### Usage

```
eigenMatMult(A, B)
```

### Arguments

A	a matrix
B	a matrix

### Value

The matrix product of A times B

### Examples

```
A = matrix(0, ncol = 500, nrow = 500)
system.time(A %% A)
system.time(eigenMatMult(A, A))
```

---

eigenResidop	<i>fast matrix residual operator using RcppEigen</i>
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---

**Description**

fast matrix residual operator using RcppEigen

**Usage**

```
eigenResidop(A, B)
```

**Arguments**

A	a matrix
B	a matrix

**Value**

The matrix product of

$$A - B(B^t B)^{-1} B^t A$$

**Examples**

```
Y = M = diag(1, 500)
system.time(scMerge::eigenResidop(Y, M))
system.time(ruv::residop(Y, M))
```

---

example_sce	<i>Subsetted mouse ESC 'SingleCellExperiment' object</i>
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---

**Description**

A dataset containing 300 cells and 2026 genes from two batches of mouse ESC data

**Usage**

```
data(example_sce, package = 'scMerge')
```

**Format**

A 'SingleCellExperiment' object

**Source**

<https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-2600/>

**References**

Kolodziejczyk et al.

fastRUVIII

*A fast version of the ruv::RUVIII algorithm***Description**

Perform a fast version of the ruv::RUVIII algorithm for scRNA-Seq data noise estimation

**Usage**

```
fastRUVIII(
  Y,
  M,
  ctl,
  k = NULL,
  eta = NULL,
  svd_k = 50,
  include.intercept = TRUE,
  average = FALSE,
  BPPARAM = SerialParam(),
  BSPARAM = ExactParam(),
  fullalpha = NULL,
  return.info = FALSE,
  inputcheck = TRUE
)
```

**Arguments**

Y	The unnormalised scRNA-Seq data matrix. A m by n matrix, where m is the number of observations and n is the number of features.
M	The replicate mapping matrix. The mapping matrix has m rows (one for each observation), and each column represents a set of replicates. The (i, j)-th entry of the mapping matrix is 1 if the i-th observation is in replicate set j, and 0 otherwise. See ruv::RUVIII for more details.
ctl	An index vector to specify the negative controls. Either a logical vector of length n or a vector of integers.
k	The number of unwanted factors to remove. This is inherited from the ruvK argument from the scMerge::scMerge function.
eta	Gene-wise (as opposed to sample-wise) covariates. See ruv::RUVIII for details.
svd_k	If BSPARAM is set to RandomParam or Ir1baParam class from BiocSingular package, then svd_k will be used to reduce the computational cost of singular value decomposition. Default to 50.
include.intercept	When eta is specified (not NULL) but does not already include an intercept term, this will automatically include one. See ruv::RUVIII for details.
average	Average replicates after adjustment. See ruv::RUVIII for details.
BPPARAM	A BiocParallelParam class object from the BiocParallel package is used. Default is SerialParam().
BSPARAM	A BiocSingularParam class object from the BiocSingular package is used. Default is ExactParam().

fullalpha	Not used. Please ignore. See ruv::RUVIII for details.
return.info	Additional information relating to the computation of normalised matrix. We recommend setting this to true.
inputcheck	We recommend setting this to true.

**Value**

A normalised matrix of the same dimensions as the input matrix Y.

**Author(s)**

Yingxin Lin, John Ormerod, Kevin Wang

**Examples**

```
L = ruvSimulate(m = 200, n = 500, nc = 400, nCelltypes = 3, nBatch = 2, lambda = 0.1, sce = FALSE)
Y = L$Y; M = L$M; ctl = L$ctl
improved1 = scMerge::fastRUVIII(Y = Y, M = M, ctl = ctl,
k = 20, BSPARAM = BiocSingular::ExactParam())
improved2 = scMerge::fastRUVIII(Y = Y, M = M, ctl = ctl,
k = 20, BSPARAM = BiocSingular::RandomParam(), svd_k = 50)
old = ruv::RUVIII(Y = Y, M = M, ctl = ctl, k = 20)
all.equal(improved1, old)
all.equal(improved2, old)
```

---

ruvSimulate	<i>Simulate a simple matrix or SingleCellExperiment to test internals of scMerge</i>
-------------	--

---

**Description**

This function is designed to generate Poisson-random-variable data matrix to test on the internal algorithms of scMerge. It does not represent real biological situations and it is not intended to be used by end-users.

**Usage**

```
ruvSimulate(
  m = 100,
  n = 5000,
  nc = floor(n/2),
  nCelltypes = 3,
  nBatch = 2,
  k = 20,
  lambda = 0.1,
  sce = FALSE
)
```

**Arguments**

m	Number of observations
n	Number of features
nc	Number of negative controls
nCelltypes	Number of cell-types
nBatch	Number of batches
k	Number of unwanted factors in simulation
lambda	Rate parameter for random Poisson generation
sce	If TRUE, returns a SingleCellExperiment object

**Value**

If sce is FALSE, then the output is a list consists of

- Y, expression matrix generated through Poisson random variables,
- ctl, a logical vector indicating the control genes,
- M, replicate mapping matrix,
- cellTypes, a vector indicating simulated cell types
- batch, a vector indicating simulated batches

if sce is TRUE, a SingleCellExperiment wrapper will be applied on all above simulated objects.

**Examples**

```
set.seed(1)
L = ruvSimulate(m = 200, n = 1000, nc = 200,
nCelltypes = 3, nBatch = 2, lambda = 0.1, k = 10, sce = TRUE)
print(L)
example <- scMerge(sce_combine = L,
                   ctl = paste0('gene', 1:500),
                   cell_type = L$cellTypes,
                   ruvK = 10,
                   assay_name = 'scMerge')
L = scater::runPCA(L, exprs_values = "logcounts")
scater::plotPCA(L, colour_by = 'cellTypes', shape = 'batch')

example = scater::runPCA(example, exprs_values = 'scMerge')
scater::plotPCA(example, colour_by = 'cellTypes', shape = 'batch')
```

---

sce\_cbind

*Combine several SingleCellExperiment objects from different batches/experiments*

---

**Description**

Combine several SingleCellExperiment objects from different batches/experiments.

**Usage**

```
sce_cbind(
  sce_list,
  method = "intersect",
  cut_off_batch = 0.01,
  cut_off_overall = 0.01,
  exprs = c("counts", "logcounts"),
  colData_names = NULL,
  batch_names = NULL
)
```

**Arguments**

sce_list	A list contains the SingleCellExperiment Object from each batch
method	A string indicates the method of combining the gene expression matrix, either union or intersect. Default to intersect. union only supports matrix class.
cut_off_batch	A numeric vector indicating the cut-off for the proportion of a gene is expressed within each batch
cut_off_overall	A numeric vector indicating the cut-off for the proportion of a gene is expressed overall data
exprs	A string vector indicating the expression matrices to be combined. The first assay named will be used to determine the proportion of zeroes.
colData_names	A string vector indicating the colData that are combined
batch_names	A string vector indicating the batch names for the output sce object

**Value**

A SingleCellExperiment object with the list of SCE objects combined.

**Author(s)**

Yingxin Lin

**Examples**

```
data('example_sce', package = 'scMerge')
batch_names<-unique(example_sce$batch)
sce_list<-list(example_sce[,example_sce$batch=='batch2'],
               example_sce[,example_sce$batch=='batch3'])
sce_combine<-sce_cbind(sce_list,batch_names=batch_names)
```

---

scMerge

*Perform the scMerge algorithm*

---

**Description**

Merge single-cell RNA-seq data from different batches and experiments leveraging (pseudo)-replicates and control genes.

**Usage**

```

scMerge(
  sce_combine,
  ctl = NULL,
  kmeansK = NULL,
  exprs = "logcounts",
  hvg_exprs = "counts",
  batch_name = "batch",
  marker = NULL,
  marker_list = NULL,
  ruvK = 20,
  replicate_prop = 1,
  cell_type = NULL,
  cell_type_match = FALSE,
  cell_type_inc = NULL,
  BSPARAM = ExactParam(),
  svd_k = 50,
  dist = "cor",
  WV = NULL,
  WV_marker = NULL,
  BPPARAM = SerialParam(),
  return_all_RUV = FALSE,
  BACKEND = NULL,
  assay_name = NULL,
  plot_igraph = TRUE,
  verbose = FALSE
)

```

**Arguments**

sce_combine	A SingleCellExperiment object contains the batch-combined matrix with batch info in colData.
ctl	A character vector of negative control. It should have a non-empty intersection with the rows of sce_combine.
kmeansK	A vector indicates the kmeans's K for each batch. The length of kmeansK needs to be the same as the number of batch.
exprs	A string indicating the name of the assay requiring batch correction in sce_combine, default is logcounts.
hvg_exprs	A string indicating the assay that to be used for highly variable genes identification in sce_combine, default is counts.
batch_name	A character indicating the name of the batch column, default to "batch"
marker	An optional vector of markers, to be used in calculation of mutual nearest cluster. If no markers input, highly variable genes will be used instead.
marker_list	An optional list of markers for each batch, which will be used in calculation of mutual nearest cluster.
ruvK	An optional integer/vector indicating the number of unwanted variation factors that are removed, default is 20.
replicate_prop	A number indicating the ratio of cells that are included in pseudo-replicates, ranges from 0 to 1. Default to 1.



cell_type	An optional vector indicating the cell type information for each cell in the batch-combined matrix. If it is NULL, pseudo-replicate procedure will be run to identify cell type.
cell_type_match	An optional logical input for whether to find mutual nearest cluster using cell type information.
cell_type_inc	An optional vector indicating the indices of the cells that will be used to super-vise the pseudo-replicate procedure.
BSPARAM	A BiocSingularParam class object from the BiocSingular package is used. Default is ExactParam().
svd_k	If BSPARAM is set to RandomParam or IrlbaParam class from BiocSingular package, then svd_k will be used to reduce the computational cost of singular value decomposition. Default to 50.
dist	The distance metrics that are used in the calculation of the mutual nearest cluster, default is Pearson correlation.
WV	A optional vector indicating the wanted variation factor other than cell type info, such as cell stages.
WV_marker	An optional vector indicating the markers of the wanted variation.
BPPARAM	A BiocParallelParam class object from the BiocParallel package is used. Default is SerialParam().
return_all_RUV	If FALSE, then only returns a SingleCellExperiment object with original data and one normalised matrix. Otherwise, the SingleCellExperiment object will contain the original data and one normalised matrix for each ruvK value. In this latter case, assay_name must have the same length as ruvK.
BACKEND	The BACKEND parameter used in DelayedArray::realize(result, BACKEND = BACKEND) to coerce the final output. Default to NULL, see DelayedArray::supportedRealizationBackends() for other options.
assay_name	The assay name(s) for the adjusted expression matrix(matrices). If return_all_RUV = TRUE assay_name must have the same length as ruvK.
plot_igraph	If TRUE, then during the un/semi-supervised scMerge, igraph plot will be displayed
verbose	If TRUE, then all intermediate steps will be shown. Default to FALSE.

### Value

Returns a SingleCellExperiment object with following components:

- assays: the original assays and also the normalised matrix
- metadata: containing the ruvK vector, ruvK\_optimal based on F-score, and the replicate matrix

### Author(s)

Yingxin Lin, Kevin Wang

**Examples**

```
## Loading example data
data('example_sce', package = 'scMerge')
## Previously computed stably expressed genes
data('segList_ensemblGeneID', package = 'scMerge')
## Running an example data with minimal inputs
sce_mESC <- scMerge(sce_combine = example_sce,
  ct1 = segList_ensemblGeneID$mouse$mouse_scSEG,
  kmeansK = c(3, 3),
  assay_name = 'scMerge')

sce_mESC = scater::runPCA(sce_mESC, exprs_values = "logcounts")
scater::plotPCA(sce_mESC, colour_by = 'cellTypes', shape = 'batch')

sce_mESC = scater::runPCA(sce_mESC, exprs_values = 'scMerge')
scater::plotPCA(sce_mESC, colour_by = 'cellTypes', shape = 'batch')
```

---

scReplicate

---

*Create replicate matrix for scMerge algorithm*


---

**Description**

Create replicate matrix for scMerge algorithm using un-/semi-/supervised approaches.

**Usage**

```
scReplicate(
  sce_combine,
  batch = NULL,
  kmeansK = NULL,
  exprs = "logcounts",
  hvg_exprs = "counts",
  marker = NULL,
  marker_list = NULL,
  replicate_prop = 1,
  cell_type = NULL,
  cell_type_match = FALSE,
  cell_type_inc = NULL,
  dist = "cor",
  WV = NULL,
  WV_marker = NULL,
  BPPARAM = SerialParam(),
  return_all = FALSE,
  BSPARAM = ExactParam(),
  plot_igraph = TRUE,
  verbose = FALSE
)
```

**Arguments**

`sce_combine` A SingleCellExperiment object contains the batch-combined matrix with batch info in colData

batch	A vector indicates the batch information for each cell in the batch-combined matrix.
kmeansK	A vector indicates the kmeans's K for each batch, length of kmeansK needs to be the same as the number of batch.
exprs	A string indicates the assay that are used for batch correction, default is log-counts
hvg_exprs	A string indicates the assay that are used for highly variable genes identification, default is counts
marker	A vector of markers, which will be used in calculation of mutual nearest cluster. If no markers input, highly variable genes will be used instead
marker_list	A list of markers for each batch, which will be used in calculation of mutual nearest cluster.
replicate_prop	A number indicating the ratio of cells that are included in pseudo-replicates, ranges from 0 to 1. Default to 1.
cell_type	A vector indicates the cell type information for each cell in the batch-combined matrix. If it is NULL, pseudo-replicate procedure will be run to identify cell type.
cell_type_match	Whether find mutual nearest cluster using cell type information
cell_type_inc	A vector indicates the indices of the cells that will be used to supervise the pseudo-replicate procedure
dist	The distance metrics that are used in the calculation of the mutual nearest cluster, default is Pearson correlation.
WV	A vector indicates the wanted variation factor other than cell type info, such as cell stages.
WV_marker	A vector indicates the markers of the wanted variation.
BPPARAM	A BiocParallelParam class object from the BiocParallel package is used. Default is SerialParam().
return_all	If FALSE, only return the replicate matrix.
BSPARAM	A BiocSingularParam class object from the BiocSingular package is used. Default is ExactParam().
plot_igraph	If TRUE, then during the un/semi-supervised scMEdge, igraph plot will be displayed
verbose	If TRUE, then all intermediate steps will be shown. Default to FALSE.

### Value

If return\_all is FALSE, return a replicate matrix. If return\_sce is TRUE, return the followings

repMat	replicate matrix
mnc	mutual nearest cluster
replicate vector	replicate vector
HVG	highly variable genes used in scReplicate

A cell-replicates mapping matrix. Each row correspond to a cell from the input expression matrix, and each column correspond to a cell-cluster/cell-type. An element of the mapping matrix is 1 if the scReplicate algorithm determines that this cell should belong to that cell cluster and 0 otherwise.

**Author(s)**

Yingxin Lin, Kevin Wang

**Examples**

```
## Loading example data
set.seed(1)
data('example_sce', package = 'scMerge')
scRep_result = scReplicate(
  sce_combine = example_sce,
  batch = example_sce$batch,
  kmeansK = c(3,3))
```

scRUVg

*RUVg function for single cell (under development)***Description**

Modified based on RUV2 from package `ruv` and RUVg from package `RUVSeq` function (see these function's documentations for full documentations and usage)

**Usage**

```
scRUVg(
  Y,
  ctl,
  k,
  Z = 1,
  eta = NULL,
  include.intercept = TRUE,
  fullW = NULL,
  svdyc = NULL
)
```

**Arguments**

<code>Y</code>	The data. A $m$ by $n$ matrix, where $m$ is the number of observations and $n$ is the number of features.
<code>ctl</code>	index vector to specify the negative controls.
<code>k</code>	The number of unwanted factors to use.
<code>Z</code>	Any additional covariates to include in the model.
<code>eta</code>	Gene-wise (as opposed to sample-wise) covariates.
<code>include.intercept</code>	Applies to both <code>Z</code> and <code>eta</code> . When <code>Z</code> or <code>eta</code> (or both) is specified (not <code>NULL</code> ) but does not already include an intercept term, this will automatically include one. If only one of <code>Z</code> or <code>eta</code> should include an intercept, this variable should be set to <code>FALSE</code> , and the intercept term should be included manually where desired.
<code>fullW</code>	Can be included to speed up execution. Is returned by previous calls of <code>scRUVg</code>
<code>svdyc</code>	Can be included to speed up execution. For internal use; please use <code>fullW</code> instead.

**Value**

A list consists of:

- A matrix newY, the normalised matrix,
- A matrix W, the unwanted variation matrix, and ;
- A matrix alpha, this corresponding coefficient matrix for W.

**Author(s)**

Yingxin Lin, Kevin Wang

**Examples**

```
L = scMerge::ruvSimulate(m = 80, n = 1000, nc = 50, nCelltypes = 10)
Y = L$Y; ctl = L$ctl
ruvgRes = scMerge::scRUVg(Y = Y, ctl = ctl, k = 20)
```

---

scRUVIII

*scRUVIII: RUVIII algorithm optimised for single cell data*


---

**Description**

A function to perform location/scale adjustment to data as the input of RUVIII which also provides the option to select optimal RUVk according to the silhouette coefficient

**Usage**

```
scRUVIII(
  Y = Y,
  M = M,
  ctl = ctl,
  fullalpha = NULL,
  k = k,
  cell_type = NULL,
  batch = NULL,
  return_all_RUV = TRUE,
  BPPARAM = SerialParam(),
  BSPARAM = ExactParam(),
  svd_k = 50
)
```

**Arguments**

- |     |  |
|-----|--|
| Y   | The unnormalised SC data. A m by n matrix, where m is the number of observations and n is the number of features.  |
| M   | The replicate mapping matrix. The mapping matrix has m rows (one for each observation), and each column represents a set of replicates. The (i, j)-th entry of the mapping matrix is 1 if the i-th observation is in replicate set j, and 0 otherwise. See ruv::RUVIII for more details. |
| ctl | An index vector to specify the negative controls. Either a logical vector of length n or a vector of integers.   |

fullalpha	Not used. Please ignore.
k	The number of unwanted factors to remove. This is inherited from the ruvK argument from the scMerge::scMerge function.
cell_type	An optional vector indicating the cell type information for each cell in the batch-combined matrix. If it is NULL, pseudo-replicate procedure will be run to identify cell type.
batch	Batch information inherited from the scMerge::scMerge function.
return_all_RUV	Whether to return extra information on the RUV function, inherited from the scMerge::scMerge function
BPPARAM	A BiocParallelParam class object from the BiocParallel package is used. Default is SerialParam().
BSPARAM	A BiocSingularParam class object from the BiocSingular package is used. Default is ExactParam().
svd_k	If BSPARAM is set to RandomParam or IrlbaParam class from BiocSingular package, then svd_k will be used to reduce the computational cost of singular value decomposition. Default to 50.

### Value

A list consists of:

- RUV-normalised matrices: If k has multiple values, then the RUV-normalised matrices using all the supplied k values will be returned.
- optimal\_ruvK: The optimal RUV k value as determined by silhouette coefficient.

### Author(s)

Yingxin Lin, Kevin Wang

### Examples

```
L = ruvSimulate(m = 200, n = 1000, nc = 100, nCelltypes = 3, nBatch = 2, lambda = 0.1, sce = FALSE)
Y = t(log2(L$Y + 1L)); M = L$M; ctl = L$ctl; batch = L$batch;
res = scRUVIII(Y = Y, M = M, ctl = ctl, k = c(5, 10, 15, 20), batch = batch)
```

---

scSEGIndex

*Single Cell Stably Express Gene Index*

---

### Description

This function computes the single-cell Stably Expressed Gene (scSEG) index from Lin. et al. (2019) for a given single-cell count data matrix. Each gene in the data is fitted with a gamma-normal mixture model and the final SEG index is computed as an average of key parameters that measure the expression stability of a gene.

We recommend using either the pre-computed genes (see "See Also" below) or the top SEG genes from an user's own data as the control genes in the scMerge function (see the ctl argument in the scMerge function).

**Usage**

```
scSEGIndex(
  exprs_mat,
  cell_type = NULL,
  BPPARAM = SerialParam(progressbar = TRUE),
  return_all = FALSE
)
```

**Arguments**

<code>exprs_mat</code>	A log-transformed single-cell data, assumed to have no batch effect and covered a wide range of cell types. A n by m matrix, where n is the number of genes and m is the number of cells.
<code>cell_type</code>	A vector indicating the cell type information for each cell in the gene expression matrix. If it is NULL, the function calculates the scSEG index without using F-statistics.
<code>BPPARAM</code>	A <code>BiocParallelParam</code> class object from the <code>BiocParallel</code> package is used. Default is <code>SerialParam(progressbar = TRUE)</code> .
<code>return_all</code>	Default to FALSE. If set to TRUE, then all genes will be returned. Otherwise, only genes with less than 80 percent zeroes will be returned.

**Value**

Returns a data frame. Each row is a gene and each column is a statistic relating to the stability of expression of each gene. The main statistic is the `segIdx` column, which is the SEG index.

**Author(s)**

Shila Ghazanfar, Yingxin Lin, Pengyi Yang

**References**

Evaluating stably expressed genes in single cells (2019). doi:10.1093/gigascience/giz106.

**See Also**

Download human SEG directly from this [link](#); Download mouse SEG directly from this [link](#).

**Examples**

```
## Loading example data
data('example_sce', package = 'scMerge')
## subsetting genes to illustrate usage.
exprs_mat = SummarizedExperiment::assay(example_sce, 'logcounts')[1:110, 1:20]
set.seed(1)
result1 = scSEGIndex(exprs_mat = exprs_mat)
## If parallelisation is needed:
param = BiocParallel::MulticoreParam(workers = 2, progressbar = TRUE)
result2 = scSEGIndex(exprs_mat = exprs_mat, BPPARAM = param)
## Closing the parallelisation
BiocParallel::register(BPPARAM = BiocParallel::SerialParam())
```

---

segList	<i>Stably expressed gene list in official gene symbols for both human and mouse</i>
---------	---

---

**Description**

A list includes the stably expressed genes for both human and mouse

**Usage**

```
data(segList, package = 'scMerge')
```

**Format**

An object of class list of length 2.

---

segList_ensemblGeneID	<i>Stably expressed gene list in EnsemblGeneID for both human and mouse</i>
-----------------------	---

---

**Description**

A list includes the stably expressed genes for both human and mouse

**Usage**

```
data(segList_ensemblGeneID, package = 'scMerge')
```

**Format**

An object of class list of length 2.



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