

# Package ‘MLSeq’

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**Description** This package applies several machine learning methods, including SVM, bagSVM, Random Forest and CART to RNA-Seq data.

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**Description**

This package applies machine learning methods, such as Support Vector Machines (SVM), Random Forest (RF), Classification and Regression Trees (CART), Linear Discriminant Analysis (LDA) and more to RNA-Seq data. MLSeq combines well-known differential expression algorithms from bioconductor packages with functions from a famous package `caret`, which has comprehensive machine learning algorithms for classification and regression tasks. Although `caret` has 200+ classification/regression algorithm built-in, approximately 85 classification algorithms are used in MLSeq for classifying gene-expression data. See `availableMethods()` for further information.

**Author(s)**

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**See Also**

[availableMethods](#), [getModelInfo](#)

Package: MLSeq  
Type: Package  
License: GPL (>= 2)

---

`Available-classifiers` *Available classification/regression methods in MLSeq*

---

**Description**

This function returns a character vector of available classification/regression methods in MLSeq. These methods are imported from `caret` package. See details below.

**Usage**

```
availableMethods(model = NULL, regex = TRUE, ...)
```

```
printAvailableMethods()
```

**Arguments**

model	a character string indicating the name of classification model. If NULL, all the available methods from MLSeq is returned. Otherwise, the methods which are complete or partial matches to requested string is returned. See <code>regex</code> for details.
regex	a logical: should a regular expressions be used? If FALSE, a simple match is conducted against the whole name of the model.
...	options to pass to <code>grepl</code> .

**Details**

There are 200+ methods available in `caret`. We import approximately 85 methods which are available for "classification" task. Some of these methods are available for both classification and regression tasks. `availableMethods()` returns a character vector of available methods in MLSeq. These names are directly used in `classify` function with argument `method`. See <http://topepo.github.io/caret/available-models.html> for a complete list of available methods in `caret`. Run `printAvailableMethods()` to print detailed information about classification methods (prints to R Console).

**Value**

a requested or complete character vector of available methods.

**Note**

Available methods in MLSeq will be regularly updated. Some of the methods might be removed as well as some others took its place in MLSeq. Please check the available methods before fitting the model. This function is inspired from the function `getModelInfo()` in `caret` and some of the code chunks and help texts are used here.

**See Also**

`classify`, `getModelInfo`, `train`

---

cervical

*Cervical cancer data*


---

**Description**

Cervical cancer data measures the expressions of 714 miRNAs of human samples. There are 29 tumor and 29 non-tumor cervical samples and these two groups are treated as two separate classes.

**Format**

A data frame with 58 observations and 714 variables (i.e miRNAs of human samples).

**Source**

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2880020/#supplementary-material-sec>

**References**

Witten, D., et al. (2010) Ultra-high throughput sequencing-based small RNA discovery and discrete statistical biomarker analysis in a collection of cervical tumours and matched controls. *BMC Biology*, 8:58

**Examples**

```
## Not run:  
data(cervical)  
  
## End(Not run)
```

---

classify

*Fitting classification models to sequencing data*

---

**Description**

This function fits classification algorithms to sequencing data and measures model performances using various statistics.

**Usage**

```
classify(  
  data,  
  method = "rpart",  
  B = 25,  
  ref = NULL,  
  class.labels = NULL,  
  preProcessing = c("deseq-vst", "deseq-rlog", "deseq-logcpm", "tmm-logcpm", "logcpm"),  
  normalize = c("deseq", "TMM", "none"),  
  control = NULL,  
  ...  
)
```

**Arguments**

data	a DESeqDataSet object, see the constructor functions <a href="#">DESeqDataSet</a> , <a href="#">DESeqDataSetFromMatrix</a> , <a href="#">DESeqDataSetFromHTSeqCount</a> in DESeq2 package.
method	a character string indicating the name of classification method. Methods are implemented from the caret package. Run <code>availableMethods()</code> for a list of available methods.
B	an integer. It is the number of bootstrap samples for bagging classifiers, for example "bagFDA" and "trebag". Default is 25.

ref	a character string indicating the user defined reference class. Default is NULL. If NULL is selected, first category of class labels is used as reference.
class.labels	a character string indicating the column name of colData(...). Should be given as "character". The column from colData() which matches with given column name is used as class labels of samples. If NULL, first column is used as class labels. Default is NULL.
preProcessing	a character string indicating the name of the preprocessing method. This option consists both the normalization and transformation of the raw sequencing data. Available options are: <ul style="list-style-type: none"> <li>• <code>deseq-vst</code>: Normalization is applied with <code>deseq</code> median ratio method. Variance stabilizing transformation is applied to the normalized data.</li> <li>• <code>deseq-rlog</code>: Normalization is applied with <code>deseq</code> median ratio method. Regularized logarithmic transformation is applied to the normalized data.</li> <li>• <code>deseq-logcpm</code>: Normalization is applied with <code>deseq</code> median ratio method. Log of counts-per-million transformation is applied to the normalized data.</li> <li>• <code>tmm-logcpm</code>: Normalization is applied with trimmed mean of M values (TMM) method. Log of counts-per-million transformation is applied to the normalized data.</li> <li>• <code>logcpm</code>: Normalization is not applied. Log of counts-per-million transformation is used for the raw counts.</li> </ul> <p><b>IMPORTANT:</b> See Details for further information.</p>
normalize	a character string indicating the type of normalization. Should be one of 'deseq', 'tmm' and 'none'. Default is 'deseq'. This option should be used with discrete and voom-based classifiers since no transformation is applied on raw counts. For caret-based classifiers, the argument 'preProcessing' should be used.
control	a list including all the control parameters passed to model training process. This argument should be defined using wrapper functions <code>trainControl</code> for caret-based classifiers, <code>discreteControl</code> for discrete classifiers (PLDA, PLDA2 and NBLDA) and <code>voomControl</code> for voom-based classifiers ( <code>voomDLDA</code> , <code>voomDQDA</code> and <code>voomNSC</code> ). See related functions for further details.
...	optional arguments passed to selected classifiers.

## Details

MLSeq consists both microarray-based and discrete-based classifiers along with the preprocessing approaches. These approaches include both normalization techniques, i.e. `deseq` median ratio (Anders et al., 2010) and trimmed mean of M values (Robinson et al., 2010) normalization methods, and the transformation techniques, i.e. variance-stabilizing transformation (`vst`) (Anders and Huber, 2010), regularized logarithmic transformation (`rlog`) (Love et al., 2014), logarithm of counts per million reads (`log-cpm`) (Robinson et al., 2010) and variance modeling at observational level (`voom`) (Law et al., 2014). Users can directly upload their raw RNA-Seq count data, preprocess their data, build one of the numerous classification models, optimize the model parameters and evaluate the model performances.

MLSeq package consists of a variety of classification algorithms for the classification of RNA-Seq data. These classifiers are categorized into two class: i) microarray-based classifiers after proper transformation, ii) discrete-based classifiers. First option is to transform the RNA-Seq data to bring

it hierarchically closer to microarrays and apply microarray-based algorithms. These methods are implemented from the caret package. Run `availableMethods()` for a list of available methods. Note that voom transformation both exports transformed gene-expression matrix as well as the precision weight matrices in same dimension. Hence, the classifier should consider these two matrices. Zararsiz (2015) presented voom-based diagonal discriminant classifiers and the sparse voom-based nearest shrunken centroids classifier. Second option is to build new discrete-based classifiers to classify RNA-Seq data. Two methods are currently available in the literature. Witten (2011) considered modeling these counts with Poisson distribution and proposed sparse Poisson linear discriminant analysis (PLDA) classifier. The authors suggested a power transformation to deal with the overdispersion problem. Dong et al. (2016) extended this approach into a negative binomial linear discriminant analysis (NBLDA) classifier. More detailed information can be found in referenced papers.

**Value**

an MLSeq object for trained model.

**Author(s)**

Dincer Goksuluk, Gokmen Zararsiz, Selcuk Korkmaz, Vahap Eldem, Ahmet Ozturk and Ahmet Ergun Karaagaoglu

**References**

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- Dong et al. (2016). NBLDA: negative binomial linear discriminant analysis for rna-seq data. *BMC Bioinformatics*, 17(1):369, Sep 2016. doi: 10.1186/s12859-016-1208-1.
- Zararsiz G (2015). Development and Application of Novel Machine Learning Approaches for RNA-Seq Data Classification. PhD thesis, Hacettepe University, Institute of Health Sciences, June 2015.

**See Also**

[predictClassify](#), [train](#), [trainControl](#), [voomControl](#), [discreteControl](#)

**Examples**

```

## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                     colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies
## 1. caret-based classifiers:
# Random Forest (RF) Classification
rf <- classify(data = data.trainS4, method = "rf",
              preprocessing = "deseq-vst", ref = "T",
              control = trainControl(method = "repeatedcv", number = 5,
                                    repeats = 2, classProbs = TRUE))
rf

# 2. Discrete classifiers:
# Poisson Linear Discriminant Analysis
pmodel <- classify(data = data.trainS4, method = "PLDA", ref = "T",
                  class.labels = "condition", normalize = "deseq",
                  control = discreteControl(number = 5, repeats = 2,
                                             tuneLength = 10, parallel = TRUE))
pmodel

# 3. voom-based classifiers:
# voom-based Nearest Shrunken Centroids
vmodel <- classify(data = data.trainS4, normalize = "deseq", method = "voomNSC",
                  class.labels = "condition", ref = "T",
                  control = voomControl(number = 5, repeats = 2, tuneLength = 10))
vmodel

## End(Not run)

```



---

confusionMat	<i>Accessors for the 'confusionMat' slot.</i>
--------------	---

---

### Description

This slot stores the confusion matrix for the trained model using `classify` function.

### Usage

```
confusionMat(object)

## S4 method for signature 'MLSeq'
confusionMat(object)

## S4 method for signature 'MLSeqModelInfo'
confusionMat(object)
```

### Arguments

`object` an `MLSeq` or `MLSeqModelInfo` object.

### Details

`confusionMat` slot includes information about cross-tabulation of observed and predicted classes and corresponding statistics such as accuracy rate, sensitivity, specificity, etc. The returned object is in `confusionMatrix` class of `caret` package. See [confusionMatrix](#) for details.

### See Also

[confusionMatrix](#)

### Examples

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
```

```

nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
                ref = "T", preProcessing = "deseq-vst",
                control = trainControl(method = "repeatedcv", number = 5,
                                       repeats = 3, classProbs = TRUE))

confusionMat(cart)

## End(Not run)

```

---

control

*Accessors for the 'control' slot.*


---

### Description

This slot stores the information about control parameters of selected classification model.

### Usage

```
control(object)
```

```
control(object) <- value
```

```
## S4 method for signature 'MLSeq'
```

```
control(object)
```

```
## S4 replacement method for signature 'MLSeq,list'
```

```
control(object) <- value
```

### Arguments

**object** an MLSeq or MLSeqModelInfo object.

**value** a list with elements for controlling trained model. It should be a list returned from one of [discreteControl](#), [voomControl](#), [trainControl](#) functions.

**See Also**

[discreteControl](#), [voomControl](#), [trainControl](#)

**Examples**

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

control(cart)

## End(Not run)
```

---

discrete.train-class    discrete.train *object*

---

**Description**

This object is the subclass for the `MLSeq.train` class. It contains trained model information for discrete classifiers such as Poisson Linear Discriminant Analysis (PLDA) and Negative Binomial Linear Discriminant Analysis (NBLDA).

**Slots**

**inputs:** a list with elements used as input for classification task.

**control:** a list with control parameters for discrete classifiers, e.g. PLDA, PLDA2 and NBLDA.

**crossValidatedModel:** a list. It stores the results for cross validation.

**finalModel:** a list. This is the trained model with optimum parameters.

**tuningResults:** a list. It stores the results for tuning parameter if selected classifier has one or more parameters to be optimized.

**callInfo:** a list. call info for selected method.

---

discreteControl	<i>Define controlling parameters for discrete classifiers (NBLDA and PLDA)</i>
-----------------	--

---

**Description**

This function sets the control parameters for discrete classifiers (PLDA and NBLDA) while training the model.

**Usage**

```
discreteControl(
  method = "repeatedcv",
  number = 5,
  repeats = 10,
  rho = NULL,
  rhos = NULL,
  beta = 1,
  prior = NULL,
  alpha = NULL,
  truephi = NULL,
  foldIdx = NULL,
  tuneLength = 30,
  parallel = FALSE,
  ...
)
```

**Arguments**

method	validation method. Support repeated cross validation only ("repeatedcv").
number	a positive integer. Number of folds.
repeats	a positive integer. Number of repeats.
rho	a single numeric value. This parameter is used as tuning parameter in PLDA classifier. It does not effect NBLDA classifier.

rhos	a numeric vector. If optimum parameter is searched among given values, this option should be used.
beta	parameter of Gamma distribution. See PLDA for details.
prior	prior probabilities of each class. a numeric vector.
alpha	a numeric value in the interval 0 and 1. It is used to apply power transformation through PLDA method.
truephi	a numeric value. If true value of genewise dispersion is known and constant for all genes, this parameter should be used.
foldIdx	a list including the fold indexes. Each element of this list is the vector indices of samples which are used as test set in this fold.
tuneLength	a positive integer. If there is a tuning parameter in the classifier, this value is used to define total number of tuning parameter to be searched.
parallel	if TRUE, parallel computing is performed.
...	further arguments. Deprecated.

**Author(s)**

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**See Also**

[classify](#), [trainControl](#), [discreteControl](#)

**Examples**

```
1L
```

---

input	<i>Accessors for the 'inputObject' slot of an MLSeq object</i>
-------	--

---

**Description**

MLSeq package benefits from DESeqDataSet structure from bioconductor package DESeq2 for storing gene expression data in a comprehensive structure. This object is used as an input for classification task through [classify](#). The input is stored in inputObject slot of MLSeq object.

**Usage**

```
input(object)

## S4 method for signature 'MLSeq'
input(object)
```

**Arguments**

object            an MLSeq object.

**See Also**

[classify](#), [DESeqDataSet](#)

**Examples**

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

input(cart)

## End(Not run)
```

---

isUpdated	<i>Checks if MLSeq object is updated/modified or not.</i>
-----------	---

---

**Description**

These functions are used to check whether the MLSeq object is modified and/or updated. It is possible to update classification parameters of MLSeq object which is returned by `classify()` function.

**Usage**

```
isUpdated(object)

isUpdated(object) <- value

isModified(object)

isModified(object) <- value

## S4 method for signature 'MLSeq'
isUpdated(object)

## S4 replacement method for signature 'MLSeq,logical'
isUpdated(object) <- value

## S4 method for signature 'MLSeq'
isModified(object)

## S4 replacement method for signature 'MLSeq,logical'
isModified(object) <- value
```

**Arguments**

object	an MLSeq object.
value	a logical. Change the state of update info.

**Value**

a logical.

**Examples**

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]
```

```

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

isUpdated(cart)
isModified(cart)

## End(Not run)

```

---

metaData

*Accessors for the 'metaData' slot of an MLSeq object*

---

## Description

This slot stores metadata information of MLSeq object.

## Usage

```
metaData(object)
```

```
## S4 method for signature 'MLSeq'
metaData(object)
```

## Arguments

object            an MLSeq object.



**Examples**

```

## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

metaData(cart)

## End(Not run)

```

---

method

*Accessors for the 'method'.*


---

**Description**

This slot stores the name of selected model which is used in `classify` function. The trained model is stored in slot `trainedModel`. See [trained](#) for details.

**Usage**

```
method(object)

method(object) <- value

## S4 method for signature 'MLSeq'
method(object)

## S4 method for signature 'MLSeqModelInfo'
method(object)

## S4 replacement method for signature 'MLSeq,character'
method(object) <- value
```

**Arguments**

object	an MLSeq object.
value	a character string. One of the available classification methods to replace with current method stored in MLSeq object.

**Details**

method slot stores the name of the classification method such as "svmRadial" for Radial-based Support Vector Machines, "rf" for Random Forests, "vroomNSC" for vroom-based Nearest Shrunken Centroids, etc. For the complete list of available methods, see [printAvailableMethods](#) and [availableMethods](#).

**See Also**

[trained](#)

**Examples**

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)
```

```

# train set
data.train <- data[ , -ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

method(cart)

## End(Not run)

```

---

MLSeq-class

MLSeq *object*


---

## Description

For classification, this is the main class for the MLSeq package. It contains all the information including trained model, selected genes, cross-validation results, etc.

## Details

Objects can be created by calls of the form `new("MLSeq", ...)`. This type of objects is created as a result of `classify` function of MLSeq package. It is then used in `predict` or `predictClassify` function for predicting the class labels of new samples.

## Slots

`inputObject`: stores the data in `DESeqDataSet` object.

`modelInfo`: stores all the information about classification model. The object is from subclass `MLSeqModelInfo`. See `MLSeqModelInfo-class` for details.

`metaData`: metadata for MLSeq object. The object is from subclass `MLSeqMetaData`. See `MLSeqMetaData-class` for details.

## Note

An MLSeq class stores the results of `classify` function and offers further slots that are populated during the analysis. The slot `inputObject` stores the raw and transformed data throughout the classification. The slot `modelInfo` stores all the information about classification model. These results

may contain the classification table and performance measures such as accuracy rate, sensitivity, specificity, positive and negative predictive values, etc. It also contains information on classification method, normalization and transformation used in the classification model. Lastly, the slot `metaData` stores the information about modified or updated slots in MLSeq object.

### Author(s)

Dincer Goksuluk, Gokmen Zararsiz, Selcuk Korkmaz, Vahap Eldem, Ahmet Ozturk and Ahmet Ergun Karaagaoglu

### See Also

[MLSeqModelInfo-class](#), [MLSeqMetaData-class](#)

---

MLSeqMetaData-class    MLSeqMetaData *object*

---

### Description

This object is a subclass for the MLSeq class. It contains metadata information, i.e. information on modified and/or updated elements, raw data etc..

### Details

Objects can be created by calls of the form `new("MLSeqMetaData", ...)`. This type of objects is created as a result of `classify` function of MLSeq package. It is then used in update function for updating the object in given object.

### Slots

`updated, modified`: a logical. See notes for details.

`modified.elements`: a list containing the modified elements in MLSeq object.

`rawData.DESeqDataSet`: raw data which is used for classification.

`classLabel`: a character string indicating the name of class variable.

### Note

The function `update` is used to re-run classification task with modified elements in MLSeq object. This function is useful when one wish to perform classification task with modified options without running `classify` function from the beginning. MLSeqMetaData object is used to store information on updated and/or modified elements in MLSeq object.

If an MLSeq object is modified, i.e. one or more elements in MLSeq object is replaced using related setter functions such as `method`, `ref` etc., the slot `modified` becomes TRUE. Similarly, the slot `updated` stores the information that the MLSeq object is updated (or classification task is re-run) or not. If `updated` slot is FALSE and `modified` slot is TRUE, one should run `update` to obtain the classification results by considering the modified elements.

**See Also**

[update](#), [isUpdated](#), [isModified](#)

---

MLSeqModelInfo-class    MLSeqModelInfo *object*

---

**Description**

For classification, this is the subclass for the MLSeq class. This object contains all the information about classification model.

**Details**

Objects can be created by calls of the form `MLSeqModelInfo(...)`. This type of objects is created as a result of `classify` function of MLSeq package. It is then used in `predictClassify` function for predicting the class labels of new samples.

**Slots**

`method`, `transformation`, `normalization`: these slots store the classification method, transformation technique and normalization method respectively. See notes for details.

`preProcessing`: See [classify](#) for details.

`ref`: a character string indicating the reference category for cases (diseased subject, tumor sample, etc.)

`control`: a list with controlling parameters for classification task.

`confusionMat`: confusion table and accuracy measures for the predictions.

`trainedModel`: an object of `MLSeq.train` class. It contains the trained model. See notes for details.

`trainParameters`: a list with training parameters from final model. These parameters are used for test set before predicting class labels.

`call`: a call object for classification task.

**Note**

`method`, `transformation`, `normalization` slots give the information on classifier, transformation and normalization techniques. Since all possible pairs of transformation and normalization are not available in practice, we specify appropriate transformations and normalization techniques with `preProcessing` argument in `classify` function. Finally, the information on normalization and transformation is extracted from `preProcessing` argument.

`MLSeq.train` is a union class of `train` from `caret` package, `voom.train` and `discrete.train` from MLSeq package. See related class manuals for details.

**See Also**

[train](#), [voom.train-class](#), [discrete.train-class](#)

---

 modelInfo

*Accessors for the 'modelInfo' slot of an MLSeq object*


---

### Description

This slot stores all the information about classification model.

### Usage

```
modelInfo(object)

## S4 method for signature 'MLSeq'
modelInfo(object)
```

### Arguments

object            an MLSeq object.

### Examples

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
                ref = "T", preProcessing = "deseq-vst",
```

```

        control = trainControl(method = "repeatedcv", number = 5,
                               repeats = 3, classProbs = TRUE))

modelInfo(cart)

## End(Not run)

```

---

normalization	<i>Accessors for the 'normalization' slot.</i>
---------------	--

---

### Description

This slot stores the name of normalization method which is used while normalizing the count data such as "deseq", "tmm" or "none"

### Usage

```

normalization(object)

normalization(object) <- value

## S4 method for signature 'MLSeq'
normalization(object)

## S4 method for signature 'MLSeqModelInfo'
normalization(object)

## S4 replacement method for signature 'MLSeq,character'
normalization(object) <- value

```

### Arguments

object	an MLSeq or MLSeqModelInfo object.
value	a character string. One of the available normalization methods for voom-based classifiers.

### Examples

```

## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

```

```

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
                ref = "T", preProcessing = "deseq-vst",
                control = trainControl(method = "repeatedcv", number = 5,
                                       repeats = 3, classProbs = TRUE))

normalization(cart)

## End(Not run)

```

---

plot

*Plot accuracy results from 'MLSeq' object*


---

### Description

This generic function is used to plot accuracy results from 'MLSeq' object returned by `classify` function.

### Usage

```

## S3 method for class 'MLSeq'
plot(x, y, ...)

## S4 method for signature 'MLSeq,ANY'
plot(x, y, ...)

```

### Arguments

x	an MLSeq object returned from <code>classify</code> function.
y	this parameter is not used. Deprecated.
...	further arguments. Deprecated.



**Author(s)**

Dincer Goksuluk, Gokmen Zararsiz, Selcuk Korkmaz, Vahap Eldem, Ahmet Ozturk and Ahmet Ergun Karaagaoglu

---

predict                      *Extract predictions from classify() object*

---

**Description**

This function predicts the class labels of test data for a given model.

`predictClassify` and `predict` functions return the predicted class information along with trained model. Predicted values are given either as class labels or estimated probabilities of each class for each sample. If `type = "raw"`, as can be seen in the example below, the predictions are extracted as raw class labels. In order to extract estimated class probabilities, one should follow the steps below:

- set `classProbs = TRUE` within control argument in `classify`
- set `type = "prob"` within `predictClassify`

**Usage**

```
## S3 method for class 'MLSeq'  
predict(object, test.data, ...)  
  
predictClassify(object, test.data, ...)  
  
## S4 method for signature 'MLSeq'  
predict(object, test.data, ...)
```

**Arguments**

<code>object</code>	a model of <code>MLSeq</code> class returned by <code>classify</code>
<code>test.data</code>	a <code>DESeqDataSet</code> instance of new observations.
<code>...</code>	further arguments to be passed to or from methods. These arguments are used in <code>predict.train</code> from <code>caret</code> package.

**Value**

`MLSeqObject` an `MLSeq` object returned from `classify`. See details.

`Predictions` a data frame or vector including either the predicted class probabilities or class labels of given test data.

**Note**

`predictClassify(...)` function was used in `MLSeq` up to package version 1.14.x. This function is aliased with generic function `predict`. In the upcoming versions of `MLSeq` package, `predictClassify` function will be omitted. Default function for predicting new observations will be `predict` from version 1.16.x and later.

**Author(s)**

Dincer Goksuluk, Gokmen Zararsiz, Selcuk Korkmaz, Vahap Eldem, Ahmet Ozturk and Ahmet Ergun Karaagaoglu

**See Also**

[classify](#), [train](#), [trainControl](#)

**Examples**

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                     colData = classtr, formula(~ 1))

# test set
data.test <- data[ ,ind]
data.test <- as.matrix(data.test + 1)
classts <- data.frame(condition=class[ind, ])

data.testS4 <- DESeqDataSetFromMatrix(countData = data.test,
                                     colData = classts, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

cart
```

```
# predicted classes of test samples for CART method (class probabilities)
pred.cart = predictClassify(cart, data.testS4, type = "prob")
pred.cart

# predicted classes of test samples for RF method (class labels)
pred.cart = predictClassify(cart, data.testS4, type = "raw")
pred.cart

## End(Not run)
```

---

preProcessing

*Accessors for the 'preProcessing' slot of an MLSeq object*

---

## Description

MLSeq package benefits from DESeqDataSet structure from bioconductor package DESeq2 for storing gene expression data in a comprehensive structure. This object is used as an input for classification task through [classify](#). The input is stored in inputObject slot of MLSeq object.

## Usage

```
preProcessing(object)

preProcessing(object) <- value

## S4 method for signature 'MLSeq'
preProcessing(object)

## S4 replacement method for signature 'MLSeq,character'
preProcessing(object) <- value
```

## Arguments

object	an MLSeq object.
value	a character string. Which preProcessing should be replaced with current one?

## See Also

[classify](#), [DESeqDataSet](#)

## Examples

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
```

```

data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

preProcessing(cart)

## End(Not run)

```

---

```
print.confMat
```

```
Print method for confusion matrix
```

---

## **Description**

This function prints the confusion matrix of the model.

## **Usage**

```

## S3 method for class 'confMat'
print(x, ..., mode = x$mode, digits = max(3, getOption("digits") - 3))

## S4 method for signature 'confMat'
print(x, ..., mode = x$mode, digits = max(3, getOption("digits") - 3))

```

**Arguments**

x	an object of class <code>confMat</code>
...	further arguments to be passed to <code>print.table</code>
mode	see <a href="#">print.confusionMatrix</a>
digits	see <a href="#">print.confusionMatrix</a>

---

ref	<i>Accessors for the 'ref' slot.</i>
-----	--------------------------------------

---

**Description**

This slot stores the information about reference category. Confusion matrix and related statistics are calculated using the user-defined reference category.

**Usage**

```
ref(object)

ref(object) <- value

## S4 method for signature 'MLSeq'
ref(object)

## S4 method for signature 'MLSeqModelInfo'
ref(object)

## S4 replacement method for signature 'MLSeq,character'
ref(object) <- value
```

**Arguments**

object	an <code>MLSeq</code> or <code>MLSeqModelInfo</code> object.
value	a character string. Select reference category for class labels.

**Examples**

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))
```

```

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
                ref = "T", preprocessing = "deseq-vst",
                control = trainControl(method = "repeatedcv", number = 5,
                                       repeats = 3, classProbs = TRUE))

ref(cart)

## End(Not run)

```

---

<code>selectedGenes</code>	<i>Accessors for the 'selectedGenes'.</i>
----------------------------	---

---

### Description

This slot stores the name of selected genes which are used in the classifier. The trained model is stored in slot `trainedModel`. See [trained](#) for details.

### Usage

```

selectedGenes(object)

## S4 method for signature 'MLSeq'
selectedGenes(object)

```

### Arguments

`object`            an MLSeq object.

### See Also

[trained](#)

**Examples**

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ , -ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

selectedGenes(cart)

## End(Not run)
```

---

show

*Show method for MLSeq objects*

---

**Description**

Prints out the information from the trained model using `classify` function.

**Usage**

```
show.MLSeq(object)
```

```
## S4 method for signature 'MLSeq'  
show(object)  
  
## S4 method for signature 'MLSeqModelInfo'  
show(object)  
  
## S4 method for signature 'MLSeqMetaData'  
show(object)  
  
## S4 method for signature 'vroom.train'  
show(object)  
  
## S4 method for signature 'discrete.train'  
show(object)
```

### Arguments

object            an MLSeq object returned from `classify` function.

### See Also

[classify](#)

---

trained            *Accessors for the 'trainedModel' slot.*

---

### Description

This slot stores the trained model. This object is returned from `train` function in `caret` package. Any further request using `caret` functions is available for `trainedModel` since this object is in the same class as the returned object from `train`. See [train](#) for details.

### Usage

```
trained(object)  
  
## S4 method for signature 'MLSeq'  
trained(object)  
  
## S4 method for signature 'MLSeqModelInfo'  
trained(object)
```

### Arguments

object            an MLSeq or MLSeqModelInfo object.



**See Also**

[train.default](#), [voom.train-class](#), [discrete.train-class](#)

**Examples**

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                     colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

trained(cart)

## End(Not run)
```

---

trainParameters

*Accessors for the 'trainParameters' slot.*


---

**Description**

This slot stores the transformation and normalization parameters from train set. These parameters are used to normalize and transform test set using train set parameters.

**Usage**

```
trainParameters(object)

## S4 method for signature 'MLSeq'
trainParameters(object)

## S4 method for signature 'MLSeqModelInfo'
trainParameters(object)
```

**Arguments**

object            an MLSeq or MLSeqModelInfo object.

**Examples**

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preprocessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

trainParameters(cart)

## End(Not run)
```

---

transformation	<i>Accessors for the 'transformation' slot.</i>
----------------	---

---

### Description

This slot stores the name of transformation method which is used while transforming the count data (e.g "vst", "rlog", etc.)

### Usage

```
transformation(object)

## S4 method for signature 'MLSeq'
transformation(object)

## S4 method for signature 'MLSeqModelInfo'
transformation(object)
```

### Arguments

object            an MLSeq or MLSeqModelInfo object.

### Examples

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ , -ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
```

```

data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                     colData = classtr, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preProcessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

transformation(cart)

## End(Not run)

```

---

update

*Update MLSeq objects returned from classify()*


---

### Description

This function updates the MLSeq object. If one of the options is changed inside MLSeq object, it should be updated to pass its effects into classification results.

### Usage

```

## S3 method for class 'MLSeq'
update(object, ..., env = .GlobalEnv)

## S4 method for signature 'MLSeq'
update(object, ..., env = .GlobalEnv)

```

### Arguments

object	a model of MLSeq class returned by <code>classify</code>
...	optional arguments passed to <code>classify</code> function.
env	an environment. Define the environment where the trained model is stored.

### Value

same object as an MLSeq object returned from `classify`.

### Note

When an MLSeq object is updated, new results are updated on the given object. The results before update process are lost when update is done. To keep the results before update, one should copy the MLSeq object to a new object in global environment.

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

```
## Not run:
library(DESeq2)
data(cervical)

# a subset of cervical data with first 150 features.
data <- cervical[c(1:150), ]

# defining sample classes.
class <- data.frame(condition = factor(rep(c("N","T"), c(29, 29))))

n <- ncol(data) # number of samples
p <- nrow(data) # number of features

# number of samples for test set (30% test, 70% train).
nTest <- ceiling(n*0.3)
ind <- sample(n, nTest, FALSE)

# train set
data.train <- data[ ,-ind]
data.train <- as.matrix(data.train + 1)
classtr <- data.frame(condition = class[-ind, ])

# train set in S4 class
data.trainS4 <- DESeqDataSetFromMatrix(countData = data.train,
                                       colData = classtr, formula(~ 1))

# test set
data.test <- data[ ,ind]
data.test <- as.matrix(data.test + 1)
classts <- data.frame(condition=class[ind, ])

data.testS4 <- DESeqDataSetFromMatrix(countData = data.test,
                                       colData = classts, formula(~ 1))

## Number of repeats (repeats) might change model accuracies ##
# Classification and Regression Tree (CART) Classification
cart <- classify(data = data.trainS4, method = "rpart",
               ref = "T", preprocessing = "deseq-vst",
               control = trainControl(method = "repeatedcv", number = 5,
                                     repeats = 3, classProbs = TRUE))

cart

# Change classification model into "Random Forests" (rf)
method(cart) <- "rf"
rf <- update(cart)

rf
```

```
## End(Not run)
```

---

```
voom.train-class      voom.train object
```

---

### Description

This object is the subclass for the `MLSeq.train` class. It contains trained model information for voom based classifiers, i.e. "voomDLDA", "voomDQDA" and "voomNSC".

### Slots

`weightedStats`: a list with elements of weighted statistics which are used for training the model. Weights are calculated from voom transformation.

`foldInfo`: a list containing information on cross-validated folds.

`control`: a list with control parameters for voom based classifiers.

`tuningResults`: a list. It stores the cross-validation results for tuning parameter(s).

`finalModel`: a list. It stores results for trained model with optimum parameters.

`callInfo`: a list. call info for related function.

---

```
voomControl           Define controlling parameters for voom-based classifiers
```

---

### Description

This function sets the control parameters for voom based classifiers while training the model.

### Usage

```
voomControl(method = "repeatedcv", number = 5, repeats = 10, tuneLength = 10)
```

### Arguments

<code>method</code>	validation method. Support repeated cross validation only ("repeatedcv").
<code>number</code>	a positive integer. Number of folds.
<code>repeats</code>	a positive integer. Number of repeats.
<code>tuneLength</code>	a positive integer. If there is a tuning parameter in the classifier, this value is used to define total number of tuning parameter to be searched.

### Author(s)

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**See Also**

[classify](#), [trainControl](#), [discreteControl](#)

**Examples**

1L

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