## Package 'NBLDA'

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Type Package

Title Negative Binomial Linear Discriminant Analysis

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**Description** We proposed a package for the classification task which uses Negative Binomial distribution within Linear Discriminant Analysis (NBLDA). It is an extension of the 'PoiClaClu' package to Negative Binomial distribution. The classification algorithms are based on the papers Dong et al. (2016, ISSN: 1471-2105) and Witten, DM (2011, ISSN: 1932-6157) for NBLDA and PLDA, respectively. Although PLDA is a sparse algorithm and can be used for variable selection, the algorithm proposed by Dong et al. is not sparse. Therefore, it uses all variables in the classifier. Here, we extend Dong et al.'s algorithm to the sparse case by shrinking overdispersion towards 0 (Yu et al., 2013, ISSN: 1367-4803) and offset parameter towards 1 (as proposed by Witten DM, 2011). We support only the classification task with this version.

Imports methods, stats, graphics

Suggests knitr, PoiClaClu, sSeq

Depends ggplot2

License GPL (>= 2)

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NeedsCompilation no

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NBLDA-package

Classifying count data using Poisson/Negative Binomial linear discriminant analysis

#### Description

This package applies linear discriminant analysis using Poisson (PLDA) and Negative Binomial (NBLDA) distributions for the classification of count data, such as gene expression data from RNA-sequencing. PLDA algorithms have been proposed by Witten (2011) through an R package PoiClaClu, which is available at CRAN. Dong et al. (2016) proposed an extension of PLDA to negative Binomial distribution. However, the algorithm is not provided through an R package. Hence, we develop an R package NBLDA to make the proposed algorithm available through CRAN. Detailed information about mathematical backgrounds is available in the references given below.

## cervical

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#### References

Witten, DM (2011). Classification and clustering of sequencing data using a Poisson model. Ann. Appl. Stat. 5(4), 2493–2518. doi:10.1214/11-AOAS493.

Dong, K., Zhao, H., Tong, T., & Wan, X. (2016). NBLDA: negative binomial linear discriminant analysis for RNA-Seq data. BMC Bioinformatics, 17(1), 369. http://doi.org/10.1186/s12859-016-1208-1

#### See Also

https://CRAN.R-project.org/package=PoiClaClu

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

cervical

Cervical cancer data

## Description

Cervical cancer data measures the gene expression levels of 714 miRNAs of human samples. There are 29 tumor and 29 non-tumor cervical samples, and these two groups correspond to two separate classes.

## Format

A data frame with 58 observations and 715 variables (including the class labels).

#### Source

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2880020/

control

#### 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)

control

Accessors for the 'control' slot.

## Description

This slot stores control parameters for training NBLDA model.

## Usage

```
## S4 method for signature 'nblda'
control(object)
```

## S4 method for signature 'nblda\_trained'
control(object)

#### Arguments

object an nblda or nblda\_trained object.

#### See Also

trainNBLDA

FindBestTransform Find the Power Transformation Parameter.

#### Description

Use this function to find a constant value of alpha to be used for transforming count data. The power transformation parameter alpha, which approximately fits transformed data to the Poisson log-linear model, is selected using a grid search within the interval [0, 1].

## Usage

```
FindBestTransform(x, grid.length = 50)
```

## Arguments

х	an n-by-p data frame or matrix of count data. Samples should be in the rows.
grid.length	how many distinct points of alpha should be searched within the interval [0, 1]? Default is 50.

## Value

the value of alpha to be used within the power transformation.

#### Note

This function is copied from PoiClaClu package and modified to control the total number of grid search.

## Author(s)

Dincer Goksuluk

#### Examples

x <- counts\$x
FindBestTransform(x)</pre>

## Description

This function can be used to generate counts, e.g., RNA-Sequencing data, for both the classification and clustering purposes.

## Usage

```
generateCountData(n, p, K, param, sdsignal = 1, DE = 0.3, allZero.rm = TRUE,
tag.samples = FALSE)
```

## Arguments

n	number of samples.
р	number of variables/features.
К	number of classes.
param	overdispersion parameter. This parameter is matched with the argument size in the rnbinom function. Hence, the Negative Binomial distribution approximates to the Poisson distribution as param increases.
sdsignal	a nonzero numeric value. As sdsignal increases, the observed counts greatly differs among K classes.
DE	a numeric value within the interval [0, 1]. This is the proportion of total number of variables that is significantly different among K classes. The remaining part is assumed to be having no contribution to the discrimination function.
allZero.rm	a logical. If TRUE, the columns having all zero cells are dropped.
tag.samples	a logical. If TRUE, the row names are automatically generated using a tag for each sample such as "S1", "S2", etc.

## Value

x, xte	count data matrix for training and test set.
y, yte	class labels for training and test set.
truesf, truesfte	
	true size factors for training and test set. See Witten (2011) for more information on estimating size factors.

## Author(s)

Dincer Goksuluk

## getShrinkedDispersions

#### Examples

getShrinkedDispersions

Estimate Shrinked Overdispersions

#### Description

Use this function to shrink initial estimates of overdispersions towards a target value.

#### Usage

```
getShrinkedDispersions(obs, shrinkTarget = NULL, delta = NULL)
```

## Arguments

obs	a numeric vector. Initial dispersion estimates for each feature.
shrinkTarget	a numeric value. Initial dispersion estimates are shrunk towards this value. If NULL, target value is estimated from the initial dispersion estimates. See notes.
delta	a numeric value. This is the weight that is used within the shrinkage algorithm. If 0, no shrinkage is performed on initial values. If equals 1, initial values are forced to be shrunken to the target value. If NULL, weights are automatically estimated from the initial dispersion estimates.

## Value

a list with the elements of initial and adjusted (shrunken) dispersion estimates, shrinkage target, and weights that are used to shrink towards the target value. See the related paper for detailed information on shrinkage algorithm (Yu et al., 2013).

initial	initial dispersion estimates using the method-of-moments.
adj	shrunken dispersion estimates.
cmp	the means and variances of initial estimates.
delta	a weight used for shrinkage estimates. See Yu et al. (2013) for details.
target	shrinkage target for initial dispersion estimates.

## Note

This function is modified from the source codes of getAdjustDisp function in the sSeq Bioconductor package.

#### Author(s)

Dincer Goksuluk

#### References

Yu, D., Huber, W., & Vitek, O. (2013). Shrinkage estimation of dispersion in Negative Binomial models for RNA-seq experiments with small sample size. Bioinformatics, 29(10), 1275-1282.

#### See Also

getT, getAdjustDisp

## Examples

```
set.seed(2128)
initial <- runif(10, 0, 4)</pre>
```

getShrinkedDispersions(initial, 0) # shrink towards 0. getShrinkedDispersions(initial, 0, delta = 1) # force to shrink 0.

inputs

## Accessors for the 'input' slot.

### Description

This slot stores the input data for trained model.

## Usage

## S4 method for signature 'nblda'
inputs(object)

#### Arguments

object an nblda object.

## See Also

trainNBLDA

## nblda-class

#### Examples

nblda-class

nblda object

#### Description

This object is the main class for the NBLDA package. It stores inputs, results, and call info for the trained model.

#### Details

Objects can be created by calls of the form new("nblda", ...). This type of object is returned from trainNBLDA function of the NBLDA package. It is then used in predict function for predicting class labels of new samples.

## Slots

input: an nblda\_input object including the count matrix (or data.frame) and class labels.

result: an nblda\_trained object with elements from the cross-validated and final models.

call: a call expression.

## Author(s)

Dincer Goksuluk

## See Also

nblda\_trained, nblda\_input

nbldaControl

## Description

Define control parameters to be used within trainNBLDA function.

## Usage

```
nbldaControl(folds = 5, repeats = 2, foldIdx = NULL, rhos = NULL,
beta = 1, prior = NULL, transform = FALSE, alpha = NULL, truephi = NULL,
target = 0, phi.epsilon = 0.15, normalize.target = FALSE, delta = NULL,
multicore = FALSE, ...)
```

## Arguments

folds	A positive integer. The number of folds for k-fold model validation.
repeats	A positive integer. This is the number of repeats for k-fold model validation. If NULL, 0 or negative, it is set to 1.
foldIdx	a list with indices of hold-out samples for each fold. It should be a list where folds are nested within repeats. If NULL, folds and repeats are used to define hold-out samples.
rhos	A vector of tuning parameters that control the amount of soft thresholding per- formed. If NULL, it is automatically generated within trainNBLDA using tuneLength, i.e., the length of grid search. See details.
beta	A smoothing term. A Gamma(beta,beta) prior is used to fit the Poisson model. Recommendation is to just leave it at 1, the default value. See Witten (2011) and Dong et al. (2016) for details.
prior	A vector with a length equal to the number of classes indicates the prior class probabilities. If NULL, all classes are assumed to be equally distributed.
transform	a logical. If TRUE, count data is transformed using power transformation. If alpha is not specified the power transformation parameter is automatically calculated using a goodness-of-fit test. See Witten (2011) for details.
alpha	a numeric value within [0, 1] to be used for power transformation.
truephi	a vector with a length equal to the number of variables. Its elements represent the true overdispersion parameters for each variable. If a single value is given, it is recycled for all variables. If a vector whose length is not equal to the number of variables given, the first element of this vector is used and recycled for all variables. If NULL, estimated overdispersions are used in the classifier. See details.
target	a value for the shrinkage target of dispersion estimates. If NULL, then then a value that is small and minimizes the average squared difference is automatically used as the target value. See $getT$ for details.

phi.epsilon	a positive value for controlling the number of features whose dispersions are shrinked towards 0. See details.	
normalize.target		
	a logical. If TRUE and target is NULL, the target value is estimated using the normalized dispersion estimates. See getT for details.	
delta	a weight within the interval [0, 1] that is used while shrinking dispersions towards 0. When "delta = 0", initial dispersion estimates are forced to be shrunk to 1. Similarly, if "delta = 0", no shrinkage is performed on the initial estimates.	
multicore	a logical. If a parallel backend is loaded and available, the function runs in parallel setting for speeding up the computations.	
	further arguments passed to trainNBLDA.	

#### Details

rhos is used to control the level of sparsity, i.e., the number of variables (or features) used in the classifier. If a variable has no contribution to the discrimination function, it should be removed from the model. By setting rhos within the interval [0, Inf], it is possible to control the number of variables that are removed from the model. As the upper bound of rhos decreases towards 0, fewer variables are removed. If rhos = 0, all variables are included in the classifier.

truephi controls how the Poisson model differs from the Negative Binomial model. If overdispersion is zero, the Negative Binomial model converges to the Poisson model. Hence, the results from trainNBLDA are identical to PLDA results from Classify when truephi = 0.

phi.epsilon is a value used to shrink estimated overdispersions towards 0. The Poisson model assumes that there is no overdispersion in the observed counts. However, this is not a valid assumption in highly overdispersed count data. NBLDA performs a shrinkage on estimated overdispersions. Although the amount of shrinkage is dependent on several parameters such as delta, target, and truephi, some of the shrunken overdispersions might be very close to 0. By defining a threshold value for shrunken overdispersions, it is possible to shrink very small overdispersions towards 0. If estimated overdispersion is below phi.epsilon, it is shrunken to 0. If phi.epsilon = NULL, threshold value is set to 0. Hence, all the variables with very small overdispersion are included in the NBLDA model.

## Value

a list with all the control elements.

#### Author(s)

Dincer Goksuluk

#### References

Witten, DM (2011). Classification and clustering of sequencing data using a Poisson model. Ann. Appl. Stat. 5(4), 2493–2518. doi:10.1214/11-AOAS493.

Dong, K., Zhao, H., Tong, T., & Wan, X. (2016). NBLDA: negative binomial linear discriminant analysis for RNA-Seq data. BMC Bioinformatics, 17(1), 369. http://doi.org/10.1186/s12859-016-1208-1.

Yu, D., Huber, W., & Vitek, O. (2013). Shrinkage estimation of dispersion in Negative Binomial models for RNA-seq experiments with small sample size. Bioinformatics, 29(10), 1275-1282.

#### See Also

getT, getAdjustDisp

#### Examples

nbldaControl() # return default control parameters.

nbldaTrained Accessors for the 'crossValidated' slot.

#### Description

This slot stores the results for cross-validated model, e.g tuning results, optimum model parameters etc.

## Usage

## S4 method for signature 'nblda'
nbldaTrained(object)

## S4 method for signature 'nblda\_trained'
nbldaTrained(object)

#### Arguments

object an nblda or nblda\_trained object.

## See Also

trainNBLDA

## Description

This object is the subclass for the NBLDA package. It stores input objects, i.e., count data and class labels.

## Slots

- x: a data.frame or matrix containing the count data input for the NBLDA classifier.
- y: a vector of length equal to the number of rows of x. This is the class label of each subject. Should be either a numeric vector or factor.

#### Author(s)

Dincer Goksuluk

nblda\_trained-class nblda\_trained object

#### Description

This object is the subclass for the NBLDA package. It stores the cross-validated results and the final model.

## Slots

crossValidated: a list. Returns the results from cross-validation.

finalModel: a list with the elements from the final model that is fitted using optimum model parameters from the cross-validated model.

control: a list with controlling parameters for fitting NBLDA classifier.

#### Author(s)

Dincer Goksuluk

normalization

## Description

This slot stores the name of normalization method. Normalization is defined using type argument in trainNBLDA function.

## Usage

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

## S4 method for signature 'nblda\_trained'
normalization(object)

## Arguments

object an nblda or nblda\_trained object.

#### See Also

#### trainNBLDA

NullModel

#### Description

Fit a training set to the NBLDA model and estimate normalized counts. The related model parameters, which are used while normalizing training sets, are also returned to normalize test sets using training set parameters.

#### Usage

```
NullModel(x, type = c("mle", "deseq", "quantile", "none", "tmm"))
```

```
NullModelTest(null.out, xte = NULL)
```

## Arguments

х	an n-by-p data frame or matrix of count data. Samples should be in the rows.
type	the normalization method. See control for details.
null.out	an object returned from NullModel.
xte	an n-by-p count matrix or data frame of test set. These counts are normalized using the training set parameters.

## Value

a list with the normalized counts and the training set parameters that are used for normalizing the raw counts.

#### Note

These functions are copied from the PoiClaClu package and modified here to make "tmm" and "none" methods available.

#### Author(s)

Dincer Goksuluk

```
xte.out <- NullModelTest(x.out, xte)
xte.out$n # Normalized counts for test set using train set parameters.</pre>
```

plot

Plot Method for the nblda and nblda\_trained Classes

## Description

This function is used to generate model performance plots using ggplot2 functions.

## Usage

```
## S3 method for class 'nblda'
plot(
 х,
 у,
  ...,
  theme = c("nblda", "default"),
 metric = c("accuracy", "error", "sparsity"),
 return = c("plot", "aes")
)
## S3 method for class 'nblda_trained'
plot(
 х,
 у,
  ...,
  theme = c("nblda", "default"),
 metric = c("accuracy", "error", "sparsity"),
  return = c("plot", "aes")
)
## S4 method for signature 'nblda'
plot(
 х,
 у,
  · · · ,
  theme = c("nblda", "default"),
 metric = c("accuracy", "error", "sparsity"),
  return = c("plot", "aes")
)
## S4 method for signature 'nblda_trained'
plot(
 х,
 у,
```

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```
theme = c("nblda", "default"),
metric = c("accuracy", "error", "sparsity"),
return = c("plot", "aes")
```

## Arguments

)

х	a nblda object returned from the trainNBLDA or nblda_trained object returned from the nbldaTrained.
У	same as x and not required to be defined. If x is missing or NULL, nblda or nblda_trained object is imported from y.
	further arguments to be passed to plotting function ggplot.
theme	pre-defined plot themes. It can be defined outside plot function using the gg- plot's library. See examples.
metric	which metric should be used in the y-axis?
return	should a complete plot or a ggplot object from ggplot be returned? One may select "aes" in order to add plot layers to a returned ggplot aesthetics. See examples.

#### Value

A list of class ggplot.

## Author(s)

Dincer Goksuluk

#### See Also

ggplot

predict

```
# Externally defining plot theme
plot(fit, theme = "default") + theme_dark(base_size = 14)
# Return empty ggplot object and add layers.
plot(fit, theme = "nblda", return = "aes") +
   geom_point() + geom_line(linetype = 2)
```

predict

#### Extract predictions from NBLDA model

## Description

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

## Usage

```
## S3 method for class 'nblda'
predict(object, test.data, return = c("predictions", "everything"), ...)
## S4 method for signature 'nblda'
predict(object, test.data, return = c("predictions", "everything"), ...)
```

## Arguments

object	a nblda object returned from the trainNBLDA.
test.data	a data frame or matrix whose class labels to be predicted.
return	what should be returned? Predicted class labels or everything?
	further arguments to be passed to or from methods.

## Value

It is possible to return only predicted class labels or a list with elements which are used within prediction process. These arguments are as follows:

xte	count data for test set.
nste	normalized count data for test set.
ds	estimates of offset parameter for each variable. See notes.
discriminant	discriminant scores of each subject.
prior	prior probabilities for each class.
ytehat	predicted class labels for test set.
alpha	power transformation parameter. If no transformation is requested, it returns NULL.
type	normalization method.
dispersions	dispersion estimates of each variable.

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#### selectedFeatures

## Note

 $d_kj$  is simply used to re-parameterize the Negative Binomial mean as  $s_i*g_j*d_kj$  where  $s_i$  is the size factor for subject i,  $g_j$  is the total count of variable j and  $d_kj$  is the offset parameter for variable j at class k.

#### Author(s)

Dincer Goksuluk

## Examples

selectedFeatures Accessors for the 'selectedFeatures' slot.

#### Description

This slot, if not NULL, stores the selected features/variables for sparse model.

## Usage

```
## S4 method for signature 'nblda'
selectedFeatures(object)
```

## S4 method for signature 'nblda\_trained'
selectedFeatures(object)

#### Arguments

object an nblda or nblda\_trained object.

show

#### Value

a list of selected features info including the followings:

idx	column indices of selected features/variables
names	column names of selected features/variables if input data have pre-defined col- umn names.

## Note

If return.selected.features = FALSE within nbldaControl or all features/variables are selected and used in discrimination function, idx and names are returned NULL.

#### See Also

trainNBLDA, nblda, nblda\_trained

## Examples

show

Show Method for the S4 classes in NBLDA Package

### Description

Pretty print the objects in S4 classes on R console.

#### Usage

```
## S3 method for class 'nblda'
show(object)
## S4 method for signature 'nblda'
show(object)
```

## trainNBLDA

## S3 method for class 'nblda\_trained'
show(object)
## S4 method for signature 'nblda\_trained'
show(object)
## S3 method for class 'nblda\_input'
show(object)
## S4 method for signature 'nblda\_input'

show(object)

## Arguments

object an object of class nblda, nblda\_trained and nblda\_input to be printed.

#### Author(s)

Dincer Goksuluk

## Examples

trainNBLDA

Train Model over Different Tuning Parameters

#### Description

This function fits the Negative Binomial classifier using various model parameters and finds the best model parameter using the resampling based performance measures.

#### Usage

```
trainNBLDA(x, y, type = c("mle", "deseq", "quantile", "tmm"),
  tuneLength = 10, metric = c("accuracy", "error"), train.control = nbldaControl(), ...)
```

#### Arguments

x	a n-by-p data frame or matrix. Samples should be in the rows and variables in the columns. Used to train the classifier.
У	a vector of length n. Each element corresponds to a class label of a sample. Integer and/or factor types are allowed.
type	a character string indicating the type of normalization method within the NBLDA model. See details.
tuneLength	a positive integer. This is the total number of levels to be used while tuning the model parameter(s) in grid search.
metric	which criteria should be used while determining the best model parameter? overall accuracy or average number of misclassified samples?
train.control	a list with control parameters to be used in the NBLDA model. See nbldaControl for details.
	further arguments. Deprecated.

## Details

NBLDA is proposed to classify count data from any field, e.g., economics, social sciences, genomics, etc. In RNA-Seq studies, for example, normalization is used to adjust between-sample differences for downstream analysis. type is used to define normalization method. Available options are "mle", "deseq", "quantile", and "tmm". Since "deseq", "quantile", and "tmm" methods are originally proposed as robust methods to be used in RNA-Sequencing studies, one should carefully define normalization types. In greater detail, "deseq" estimates the size factors by dividing each sample by the geometric means of the transcript counts (Anders and Huber, 2010). "tmm" trims the lower and upper side of the data by log-fold changes to minimize the log-fold changes between the samples and by absolute intensity (Robinson and Oshlack, 2010). "quantile" is quantile normalization approach of Bullard et al. (2010). "mle" (less robust) divides total counts of each sample to the total counts (Witten, 2010). See related papers for mathematical backgrounds.

#### Value

an nblda object with following slots:

input	an nblda_input object including the raw count data and response variable. See nblda_input for details.
result	an nblda_trained object including the results from cross-validated and final models. See nblda_trained for details.
call	a call expression.

## Author(s)

Dincer Goksuluk

## trainNBLDA

#### References

Witten, DM (2011). Classification and clustering of sequencing data using a Poisson model. Ann. Appl. Stat. 5(4), 2493–2518. doi:10.1214/11-AOAS493.

Dong, K., Zhao, H., Tong, T., & Wan, X. (2016). NBLDA: negative binomial linear discriminant analysis for RNA-Seq data. BMC Bioinformatics, 17(1), 369. http://doi.org/10.1186/s12859-016-1208-1.

Anders S. Huber W. (2010). Differential expression analysis for sequence count data. Genome Biology, 11:R106

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

Robinson MD, Oshlack A (2010). A scaling normalization method for differential expression analysis of RNA-Seq data. Genome Biology, 11:R25, doi:10.1186/gb-2010-11-3-r25

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