# Package 'NBSplice'

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**Type** Package

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**Title** Negative Binomial Models to detect Differential Splicing

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**Description** The package proposes a differential splicing evaluation method
based on isoform quantification. It applies generalized linear models with
negative binomial distribution to infer changes in isoform relative
expression.

**URL** http://www.bdmg.com.ar

**License** GPL (>=2)

**Depends** R (>= 3.5), methods

**Imports** edgeR, stats, MASS, car, mppa, BiocParallel, ggplot2, reshape2

**Suggests** knitr, RUnit, BiocGenerics, BiocStyle

**Collate** 'NBSplice-package.R' 'IsoDataSet.R' 'IsoDataSet-getters.R'
'IsoDataSet-show.R' 'IsoDataSet-print.R'
'IsoDataSet-buildLowExpIdx.R' 'IsoDataSet-core.R'
'NBSpliceRes.R' 'NBSpliceRes-initialize.R'
'NBSpliceRes-constructor.R' 'NBSpliceRes-getters.R'
'NBSpliceRes-show.R' 'NBSpliceRes-print.R'
'IsoDataSet-NBTest.R' 'totalGeneCounts.R'
'IsoDataSet-initialize.R' 'IsoDataSet-constructor.R'
'NBSpliceRes-getDSGenes.R' 'NBSpliceRes-getDSResults.R'
'NBSpliceRes-getGeneResults.R' 'NBSpliceRes-plotRatiosDisp.R'
'NBSpliceRes-plotVolcano.R' 'NBSpliceRes-plotGeneResults.R'
'NBSplice-isoCounts.R' 'NBSplice-geneIso.R'
'NBSplice-designMatrix.R' 'NBSplice-myIsoDataSet.R'
'NBSplice-myDSResults.R'

**biocViews** Software, StatisticalMethod, AlternativeSplicing,
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**VignetteBuilder** knitr

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# R **topics documented:**

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| | |
|---|---|
| NBSplice-package | *NBSplice: Negative Binomial Models to detect Differential Splicing.* |

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## Description

The package proposes a differential splicing evaluation method based on isoform quantification data. It uses generalized linear models with negative binomial distribution to model changes in isoform relative expression and infer differential changes in gene splicing patterns.

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

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| | |
|---|---|
| buildData | *GLM fitting and hypothesis testing* |

---

## Description

buildData is an R function to build the data structure useful to fit a negative binomial model at the gene level. fitModel is an R function to fit a negative binomial generalized linear model in order to evaluate changes in the isoform ratio. Each isoform is considered as a factor. The model incorporates one factor related to one experimental condition with two levels.

## Usage

```
buildData(isoCounts, geneCounts, geneIso, gene, designMatrix, colName)

fitModel(myData, gene, formula, colName, test = c("F", "Chisq"), contrast)
```

## Arguments

| | |
|---|---|
| isoCounts | Matrix having the expression counts at the isoform level. Isoforms must be in rows and samples in columns. Rownames and colnames must be defined with isoform and samples names, respectively. |
| geneCounts | Matrix having the expression counts at the gene level. Genes must be in rows and samples in columns. Rownames and colnames must be defined with gene and sample names, respectively. |
| geneIso | Data.frame containing the relationship between isoforms and genes. It must contain two columns, named as 'gene_id' and 'isoform_id'. Its isoforms should be the same specified in the isoCounts matrix. |
| gene | Character indicating the name of the gene to be analyzed |
| designMatrix | Data.frame specifying metadata related to the experiment. Its rows must be the samples and experimental factors should be arranged on its columns. |

| colName | Character indicating the name of the column in the design matrix to be considered for mean expression calculations per experimental condition and differential expression test. |
|---------|----------------|
| myData | Data.frame containing the expression matrix at isoform levels, it means, isoform in rows and samples in columns. It is obtained using the buildData NBSplice method. |
| formula | Object with the formula of the GLM. |
| test | Character indicating the name of the distribution to assume for linear hypothesis statistic. Could be "F" or "chisq". |
| contrast | Character vector with the names of the two levels of the experimental factor to be contrasted. |

## Value

A data.frame ready to use by the model fitting function

A data.frame summarizing gene results.

## Note

see full example in `IsoDataSet-class`

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar> ## Data loading data(myIsoDataSet, package="NBSplice")

## Identificating Low expressed Isoforms myIsoDataSet<-buildLowExpIdx(myIsoDataSet)

##Arguments definition colName<-"condition" test<-"F"

## Differential splicing test myDSResults<-NBTest(myIsoDataSet, colName, test)

## See Also

`IsoDataSet`

Other IsoDataSet: `IsoDataSet-class`, `IsoDataSet`, `NBTest`, `buildLowExpIdx`, `designMatrix`, `geneIso`, `initialize`, `isoCountsData`, `myIsoDataSet`

## Examples

```
## Data loading
data(myIsoDataSet, package="NBSplice")

## Identificating Low expressed Isoforms
myIsoDataSet<-buildLowExpIdx(myIsoDataSet)

##Arguments definition
colName<-"condition"
test<-"F"
```

```
## Differential splicing test
myDSResults<-NBTest(myIsoDataSet, colName, test)
```

---

buildLowExpIdx                *IsoDataSet low expressed isoforms detection.*

---

### Description

buildLowExpIdx identifies low expressed isoforms and stores their indexes to facilitate their identification. Low expression is defined combining absolute and relative expression thresholds.

### Usage

```
buildLowExpIdx(object, colName = "condition", ratioThres = 0.01,
    countThres = 1, BPPARAM = bpparam())
## S4 method for signature 'IsoDataSet'
buildLowExpIdx(object, colName = "condition",
    ratioThres = 0.01, countThres = 1, BPPARAM = bpparam())
```

### Arguments

| | |
|---|---|
| object | IsoDataSet class object. |
| colName | Character indicating the name of the column in the design matrix to be considered for differential expression analysis. |
| ratioThres | Numeric indicating the minimum isoform's relative expression value admitted. If one isoform had expression lower than this threshold in at least one sample, thus it will be ignored for further analysis. |
| countThres | Numeric indicating the isoform's expression threshold. If one isoform showed a mean expression value lower than this threshold in at least one experimental condition, thus it will be ignored for further analysis. |
| BPPARAM | An optional BiocParallelParam instance defining the parallel back-end to be used during evaluation. |

### Value

IsoDataSet object.

### Note

see full example in [IsoDataSet-class](IsoDataSet-class)

### Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

[IsoDataSet](#)

Other IsoDataSet: [IsoDataSet-class](#), [IsoDataSet](#), [NBTest](#), [buildData](#), [designMatrix](#), [geneIso](#), [initialize](#), [isoCountsData](#), [myIsoDataSet](#)

## Examples

```
## Data loading
data(myIsoDataSet, package="NBSplice")

## Identification of low expressed isoforms
myIsoDataSet<-buildLowExpIdx(myIsoDataSet)
```

---

| designMatrix | *An example of a design matrix helpful to demonstrate the use of the NBSplice R package.* |
| --- | --- |

---

## Description

A data.frame representing the design matrix related to the isoCounts dataset.

## Format

A data.frame object

## Details

This data.frame complements the expression matrix provided as the [isoCounts](#) data. See [isoCounts](#) man page for information about [designMatrix](#) construction. Each row of this data.frame specifies one experiment sample.

Column with samples names.

**sample**condition Column indicating the condition of each sample.

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## Source

see [IsoDataSet-class](#)

## See Also

Other IsoDataSet: [IsoDataSet-class](#), [IsoDataSet](#), [NBTest](#), [buildData](#), [buildLowExpIdx](#), [geneIso](#), [initialize](#), [isoCountsData](#), [myIsoDataSet](#)

---

geneIso                         *An example of isoform-gene relationship specification helpful to demonstrate the use of the NBSplice R package.*

---

### Description

A dataset containing isoform-gene relationships.

### Format

A data.frame object

### Details

This data.frame complements the expression matrix provided as the isoCounts data. See isoCounts man page for information about geneIso construction. Each row of this data.frame specifies one specific and unique isoform-gene relationship.

Column with the gene names.

**gene_id isoform_id** Column with the isoform names.

### Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

### Source

see `IsoDataSet-class`

### See Also

Other IsoDataSet: `IsoDataSet-class`, `IsoDataSet`, `NBTest`, `buildData`, `buildLowExpIdx`, `designMatrix`, `initialize`, `isoCountsData`, `myIsoDataSet`

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GetDSGenes                         *Get differentially spliced genes.*

---

### Description

GetDSGenes returns the list of genes identified as differentially spliced.

### Usage

```
GetDSGenes(myNBRes, adjusted = TRUE, p.value = 0.05)

## S4 method for signature 'NBSpliceRes'
GetDSGenes(myNBRes, adjusted = TRUE, p.value = 0.05)
```

## Arguments

| | |
|---|---|
| `myNBRes` | NBSpliceRes class object. |
| `adjusted` | Logical indicating if adjusted p values should be used. |
| `p.value` | Numeric value between 0 and 1 giving the required family-wise error rate or false discovery rate. |

## Value

A character with the names of differentially spliced genes.

## Note

see full example in [NBSpliceRes-class](#)

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

[NBSpliceRes](#)

Other NBSpliceRes: [GetDSResults](#), [GetGeneResults](#), [NBSpliceRes-class](#), [NBSpliceRes-initialize](#), [NBSpliceRes](#), [myDSResults](#), [plotGeneResults](#), [plotRatiosDisp](#), [plotVolcano](#)

## Examples

```
data(myDSResults, package="NBSplice")

myDSGenes<-GetDSGenes(myDSResults)
```

---

GetDSResults *Get differential expression results of significant genes.*

---

## Description

`GetDSResults` returns the results obtained for those genes identified as differentially spliced.

## Usage

```
GetDSResults(myNBRes, adjusted = TRUE, p.value = 0.05)

## S4 method for signature 'NBSpliceRes'
GetDSResults(myNBRes, adjusted = TRUE,
    p.value = 0.05)
```

## Arguments

| | |
|---|---|
| `myNBRes` | NBSpliceRes class object. |
| `adjusted` | Logical indicating if adjusted p values should be used. |
| `p.value` | Numeric value between 0 and 1 giving the required family-wise error rate or false discovery rate. |

## Value

data.frame with the results obtainted by means of the NBTest method

## Note

see full example in [NBSpliceRes-class](NBSpliceRes-class)

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

[NBSpliceRes](NBSpliceRes)

Other NBSpliceRes: [GetDSGenes](GetDSGenes), [GetGeneResults](GetGeneResults), [NBSpliceRes-class](NBSpliceRes-class), [NBSpliceRes-initialize](NBSpliceRes-initialize), [NBSpliceRes](NBSpliceRes), [myDSResults](myDSResults), [plotGeneResults](plotGeneResults), [plotRatiosDisp](plotRatiosDisp), [plotVolcano](plotVolcano)

## Examples

```
data(myDSResults, package="NBSplice")

myDSResultsDF<-GetDSResults(myDSResults)
```

---

GetGeneResults *Get differentially spliced genes.*

---

## Description

`GetGeneResults` returns the results obtained for the specified gene.

## Usage

```
GetGeneResults(myNBRes, gene, filterLowExpIso = TRUE)

## S4 method for signature 'NBSpliceRes'
GetGeneResults(myNBRes, gene, filterLowExpIso = TRUE)
```

## Arguments

| | |
|---|---|
| myNBRes | NBSpliceRes class object. |
| gene | Character indicating the gene name. |
| filterLowExpIso | |
| | Logical indicating if lower-expression isoforms should be filtered out. |

## Value

Data.frame object with gene results.

## Note

see full example in [NBSpliceRes-class](#)

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

[NBSpliceRes](#)

Other NBSpliceRes: [GetDSGenes](#), [GetDSResults](#), [NBSpliceRes-class](#), [NBSpliceRes-initialize](#), [NBSpliceRes](#), [myDSResults](#), [plotGeneResults](#), [plotRatiosDisp](#), [plotVolcano](#)

## Examples

```
data(myDSResults, package="NBSplice")

myResults<-results(myDSResults)

## Select the first gene
gene<-myResults[,"gene"][1]

myGeneResults<-GetGeneResults(myDSResults, gene)
```

---

initialize                      *IsoDataSet object constructor.*

---

## Description

`initialize` creates an IsoDataSet object with the specified isoform expression counts.

## Usage

```
## S4 method for signature 'IsoDataSet'
initialize(.Object, isoCounts, experimentData, colName,
    geneIso, BPPARAM = bpparam())
```

**Arguments**

| | |
|---|---|
| `.Object` | IsoDataSet class object. |
| `isoCounts` | Matrix having the expression counts at the isoform level. Isoforms must be in rows and samples in columns. Rownames and colnames must be defined with isoform and samples names, respectively. |
| `experimentData` | Data.frame specifying metadata related to the experiment. Its rows must be the samples and experimental factors should be arranged on its columns. |
| `colName` | Character indicating the name of the column in the design matrix to be considered for mean expression calculations per experimental condition and differential expression test. |
| `geneIso` | Data.frame containing the relationship between isoforms and genes. It must contain two columns, named as 'gene_id' and 'isoform_id'. Its isoforms should be the same specified in the isoCounts matrix. |
| `BPPARAM` | An optional BiocParallelParam instance defining the parallel back-end to be used during evaluation. |

**Value**

IsoDataSet object.

**Note**

see full example in [IsoDataSet-class](IsoDataSet-class)

**Author(s)**

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

**See Also**

[IsoDataSet](IsoDataSet)

Other IsoDataSet: [IsoDataSet-class](IsoDataSet-class), [IsoDataSet](IsoDataSet), [NBTest](NBTest), [buildData](buildData), [buildLowExpIdx](buildLowExpIdx), [designMatrix](designMatrix), [geneIso](geneIso), [isoCountsData](isoCountsData), [myIsoDataSet](myIsoDataSet)

**Examples**

```
## Data loading
data(isoCounts, package="NBSplice")
data(designMatrix, package="NBSplice")
data(geneIso, package="NBSplice")

## Arguments definition
colName<-"condition"

## Constructor calling
myIsoDataSet<-IsoDataSet(isoCounts, designMatrix, colName, geneIso)
```

---

isoCountsData              *An example of an isoform counts matrix helpful to demonstrate the use*
                           *of the NBSplice R package.*

---

#### Description

A non-real dataset containing isoform expression counts of a synthetic dataset where several differential splicing changes were simulated and controlled.

#### Format

A data.frame object

#### Details

Isoform expression matrix where isoforms are in rows and samples in columns. Samples 'C1R1', 'C1R2', 'C1R3' and 'C1R4' are from Normal condition, whereas 'C2R1', 'C2R2', 'C2R3' and 'C2R4' are from Tumor condition. The matrix is a subset of an expression matrix obtained from a simulated RNA-seq experiment where differential splicing is controlled, used for NBSplice evaluation. The full expression matrix could be dowloaded from the GitHub respository https://github.com/gamerino/NBSpliceSuppInformation. In particular, the file expressionMatrixSim1.RData from the Data/sim1 folder was used here. The metadata information was obtained from the isoInfoSim1.RData file, stored in the same folder. For NBSplice package demonstration, a subset of 500 genes were selected doing genes<-unique(iso_info$gene_id) set.seed(12345) selectedGenes<-sample(genes, 500) isoInfo<-iso_info[iso_info$gene_id geneIso<-isoInfo[, c("gene_id", "transcript_id")] colnames(geneIso)[2]<-"isoform_id" isoCounts<-iso_cm[isoInfo$transcript_id,]

The isoCounts matrix, the geneIso and the designMatrix data.frames are provided with NB-Splice.

#### Author(s)

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#### Source

see [IsoDataSet-class](IsoDataSet-class)

#### See Also

Other IsoDataSet: [IsoDataSet-class](IsoDataSet-class), [IsoDataSet](IsoDataSet), [NBTest](NBTest), [buildData](buildData), [buildLowExpIdx](buildLowExpIdx), [designMatrix](designMatrix), [geneIso](geneIso), [initialize](initialize), [myIsoDataSet](myIsoDataSet)

---

| IsoDataSet | *IsoDataSet constructor* |
|---|---|

---

### Description

`IsoDataSet` creates an object to store expression counts at isoform and gene level, the relationship between those, the experiment data and the formula to be used for models fitting required to evaluate differential splicing.

### Usage

```
IsoDataSet(isoCounts, experimentData, colName, geneIso, BPPARAM = bpparam())
```

### Arguments

isoCounts          Matrix having the expression counts at the isoform level. Isoforms must be in rows and samples in columns. Rownames and colnames must be defined with isoform and samples names, respectively.

experimentData  Data.frame specifying metadata related to the experiment. Its rows must be the samples and experimental factors should be arranged on its columns.

colName            Character indicating the name of the column in the design matrix to be considered for differential splicing analysis.

geneIso            Data.frame containing the relationship between isoforms and genes. It must contain two columns, named as 'gene_id' and 'isoform_id'. Its isoforms should be the same specified in the isoCounts matrix.

BPPARAM          An optional BiocParallelParam instance defining the parallel back-end to be used during evaluation.

### Value

IsoDataSet object.

### Note

see full example in [IsoDataSet-class](#)

### Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com>, and Elmer A. Fernandez <efernandez@bdmg.com.ar>

### See Also

[IsoDataSet-class](#)

Other IsoDataSet: [IsoDataSet-class](#), [NBTest](#), [buildData](#), [buildLowExpIdx](#), [designMatrix](#), [geneIso](#), [initialize](#), [isoCountsData](#), [myIsoDataSet](#)

## Examples

```
## Data loading
data(isoCounts, package="NBSplice")
data(designMatrix, package="NBSplice")
data(geneIso, package="NBSplice")

## Arguments definition
colName<-"condition"

## Constructor calling
myIsoDataSet<-IsoDataSet(isoCounts, designMatrix, colName, geneIso)
```

---

IsoDataSet-class            *IsoDataSet S4 class implementation in R*

---

## Description

This S4 class represents a data set containing isoforms expresion in R.

## Slots

counts  Matrix containing expression values at the isoform level

geneCounts  Matrix with expression values at the gene level

colData  Data.frame with experiment information. Its rows should be the columns of the counts data.frame

isoGeneRel  Data.frame specifying the isoform-gene relationship

design  Formula to be used in the GLM fit and tests

lowExpIndex  Numeric indicating the positions of low expressed isoforms

## Features

1. Discover differential modifications in the splicing patterns.

2. Detect and quantify isoform relative expression changes.

3. Combine the results of both gene and isoform levels analysis.

## Functions

IsoDataSet S4 class includes the following functions:

**initialize**  Constructor of IsoDataSet objects.

**isoCounts**  Get the counts slot.

**geneCounts**  Get the geneCounts slot.

**isoGeneRel**  Get the isoGeneRel slot.

**colData**  Get the colData slot.

**designFormula** Get the design slot.

**lowExpIndex** Get the lowExpIdx slot.

**setDesign** Set the design slot.

**buildLowExpIdx** Build the index to identify low expressed isoforms.

**NBTest** Perform differential expression analysis.

## Author(s)

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## See Also

Other IsoDataSet: IsoDataSet, NBTest, buildData, buildLowExpIdx, designMatrix, geneIso, initialize, isoCountsData, myIsoDataSet

## Examples

```
## Data loading
data(isoCounts, package="NBSplice")
data(designMatrix, package="NBSplice")
data(geneIso, package="NBSplice")

## Arguments definition
colName<-"condition"

## Constructor calling
myIsoDataSet<-IsoDataSet(isoCounts, designMatrix, colName, geneIso)

## Identificating Low expressed Isoforms
myIsoDataSet<-buildLowExpIdx(myIsoDataSet)

##Arguments definition
colName<-"condition"
test<-"F"

## Differential splicing test
myDSResults<-NBTest(myIsoDataSet, colName, test)
```

---

IsoDataSet-getters *Getters for an IsoDataSet object.*

---

## Description

Obtain information about IsoDataSet slots, according to the given function call.

**Usage**

```
isoCounts(object, CPM = TRUE)

## S4 method for signature 'IsoDataSet'
isoCounts(object, CPM = TRUE)

geneCounts(object)

## S4 method for signature 'IsoDataSet'
geneCounts(object)

isoGeneRel(object)

## S4 method for signature 'IsoDataSet'
isoGeneRel(object)

expData(object)

## S4 method for signature 'IsoDataSet'
expData(object)

designFormula(object)

## S4 method for signature 'IsoDataSet'
designFormula(object)

lowExpIndex(object)

## S4 method for signature 'IsoDataSet'
lowExpIndex(object)
```

**Arguments**

| | |
|---|---|
| object | IsoDataSet class object. |
| CPM | Logical indicating if expression matrix at the isoform level should be in CPM scale. |

**Value**

According to the call one of the following objects can be returned

| | |
|---|---|
| matrix | Isoform expression matrix. |
| matrix | Gene expression matrix. |
| data.frame | Experiment information. |
| data.frame | Isoforms-gene relationships. |
| formula | Formula to be used in the GLM fit. |
| numeric | Index of low expressed isoforms. |

**Note**

see full example in `IsoDataSet-class`

**Author(s)**

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

**See Also**

`IsoDataSet`

**Examples**

```
## Data loading
data(myIsoDataSet, package="NBSplice")

## Getting isoform expression counts
myCounts<-isoCounts(myIsoDataSet)
head(myCounts)
## Data loading
data(myIsoDataSet, package="NBSplice")

## Getting gene expression counts
myGeneCounts<-geneCounts(myIsoDataSet)
head(myGeneCounts)
## Data loading
data(myIsoDataSet, package="NBSplice")

## Getting isoform-gene relationships
myIsoGeneRel<-isoGeneRel(myIsoDataSet)
head(myIsoGeneRel)
## Data loading
data(myIsoDataSet, package="NBSplice")

## Getting the design matrix
myDesignMatrix<-expData(myIsoDataSet)
myDesignMatrix
## Data loading
data(myIsoDataSet, package="NBSplice")

## Getting the model formula
myFormula<-designFormula(myIsoDataSet)
myFormula
## Data loading
data(myIsoDataSet, package="NBSplice")

## Getting index of low expressed isoforms
myLowExpIdx<-lowExpIndex(myIsoDataSet)
head(myLowExpIdx)
```

---

myDSResults                    *An example of an NBSpliceRes object.*

---

### Description

An example of an NBSpliceRes object helpful to demonstrate the use of the NBSplice R package.
An NBSpliceRes object built obtained using the code present as example in the [NBTest](#) man
page.

### Format

A NBSpliceRes object

### Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

### Source

see `NBSpliceRes-class`

### See Also

Other NBSpliceRes: `GetDSGenes`, `GetDSResults`, `GetGeneResults`, `NBSpliceRes-class`, `NBSpliceRes-initialize`,
`NBSpliceRes`, `plotGeneResults`, `plotRatiosDisp`, `plotVolcano`

---

myIsoDataSet                   *An example of an IsoDataSet object.*

---

### Description

An example of an IsoDataSet object helpful to demonstrate the use of the NBSplice R package.

### Format

An IsoDataSet object

### Details

An IsoDataSet object built obtained using the code present as example in the [IsoDataSet](#) man
page.

### Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## Source

see [IsoDataSet-class](#)

## See Also

Other IsoDataSet: [IsoDataSet-class](#), [IsoDataSet](#), [NBTest](#), [buildData](#), [buildLowExpIdx](#), [designMatrix](#), [geneIso](#), [initialize](#), [isoCountsData](#)

---

NBSpliceRes *NBSpliceRes constructor*

---

## Description

NBSpliceRes creates an object to store the results of differential expression analysis performed by the NBTest function.

## Usage

NBSpliceRes(results, lowExpIndex, contrast, dispersion)

## Arguments

| | |
|---|---|
| results | Data.frame with NBTest results of expressed isoforms. |
| lowExpIndex | Numeric indicating the positions of low expressed isoforms. |
| contrast | Character indicating the contrast used for NBTest. |
| dispersion | Numeric with the estimated gene dispersions. |

## Value

NBSpliceRes object.

## Note

see full example in [NBSpliceRes-class](#)

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com>, and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

[NBSpliceRes-class](#)

Other NBSpliceRes: [GetDSGenes](#), [GetDSResults](#), [GetGeneResults](#), [NBSpliceRes-class](#), [NBSpliceRes-initialize](#), [myDSResults](#), [plotGeneResults](#), [plotRatiosDisp](#), [plotVolcano](#)

## Examples

```
data(myDSResults, package="NBSplice")
myResults<-results(myDSResults, filter=FALSE)
myLowExpIdx<-lowExpIndex(myDSResults)
myContrast<-contrast(myDSResults)
myDispersion<-disp(myDSResults)
myNewDSResults<-NBSpliceRes(myResults, myLowExpIdx, myContrast, myDispersion)
```

---

NBSpliceRes-class         *NBSpliceRes S4 class implementation in R*

---

## Description

This S4 class represents a data set containing NBTest results results.

## Slots

results Data.frame with NBTest results of expressed isoforms.

lowExpIndex Numeric indicating the positions of low expressed isoforms.

contrast Character indicating the contrast used for NBTest.

dispersion Numeric model dispersions.

## Features

1. Explore differential splicing occurrence.
2. Explore isoform relative expression an its changes.
3. Combine the results of both gene and isoform levels analysis.

## Functions

NBSpliceRes S4 class includes the following functions:

**initialize** Constructor of NBSpliceRes objects.

**results** Gets the results slot.

**contrast** Gets the contrast slot.

**lowExpIndex** Gets the lowExpIdx slot.

**disp** Gets the dispersion slot.

**print** Shows a NBSpliceRes object.

**show** Shows a NBSpliceRes object.

**GetDSGenes** Returns the list of differentially spliced genes.

**GetDSResults** Returns the differential splicing results.

**GetGeneResults** Returns the NBSplice results for an specific gene.

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

Other NBSpliceRes: GetDSGenes, GetDSResults, GetGeneResults, NBSpliceRes-initialize, NBSpliceRes, myDSResults, plotGeneResults, plotRatiosDisp, plotVolcano

## Examples

```
data(myDSResults, package="NBSplice")
myResults<-results(myDSResults, filter=FALSE)
myLowExpIdx<-lowExpIndex(myDSResults)
myContrast<-contrast(myDSResults)

myNewDSResults<-NBSpliceRes(myResults, myLowExpIdx, myContrast)

##Getting differentially spliced genes
myDSGenes<-GetDSGenes(myDSResults)

##Getting the results for differentially spliced genes
myDSResultsDF<-GetDSResults(myDSResults)

##Getting the results for a particular differentially spliced gene
myResults<-results(myDSResults)

## Select the first gene
gene<-myResults[,"gene"][1]

myGeneResults<-GetGeneResults(myDSResults, gene)
```

---

NBSpliceRes-getters      *Getters for an NBSpliceRes object.*

---

## Description

Obtain information about NBSpliceRes slots, according to the given function call.

## Usage

```
results(object, filter = TRUE)

## S4 method for signature 'NBSpliceRes'
results(object, filter = TRUE)

contrast(object)

## S4 method for signature 'NBSpliceRes'
```

```
contrast(object)

## S4 method for signature 'NBSpliceRes'
lowExpIndex(object)

disp(object)

## S4 method for signature 'NBSpliceRes'
disp(object)
```

## Arguments

| object | NBSpliceRes class object. |
|--------|---------------------------|
| filter | Logical indicating if low expressed isoforms should be filtered out. |

## Value

according to the call one of the following objects can be returned

| data.frame | NBTest results. |
|------------|-----------------|
| numeric    | Index of low expressed isoforms. |
| character  | Experiment contrast. |
| numeric    | Estimated gene dispersion. |

## Note

see full example in [NBSpliceRes-class](NBSpliceRes-class)

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## Examples

```
data(myDSResults, package="NBSplice")

## Getting results slot filtering the low expressed isoforms
myResults<-results(myDSResults)

## Getting results slot keeping the low expressed isoforms
myResults<-results(myDSResults, filter=FALSE)
## Getting the contrast slot
myContrast<-contrast(myDSResults)
## Getting the lowExpIndex slot
myLowExpIndex<-lowExpIndex(myDSResults)

## Getting the dispersion slot
myDispersion<-disp(myDSResults)
```

---

NBSpliceRes-initialize

*NBSpliceRes object constructor.*

---

### Description

`initialize` creates an NBSpliceRes object

### Usage

```
## S4 method for signature 'NBSpliceRes'
initialize(.Object, results, lowExpIndex, contrast,
    dispersion)
```

### Arguments

| | |
|---|---|
| .Object | NBSpliceRes class object. |
| results | Data.frame with NBTest results of expressed isoforms. |
| lowExpIndex | Numeric indicating the positions of low expressed isoforms. |
| contrast | Character indicating the contrast used for NBTest. |
| dispersion | Numeric with the estimated gene dispersions. |

### Value

NBSpliceRes object.

### Note

see full example in [NBSpliceRes-class](#)

### Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

### See Also

[NBSpliceRes](#)

Other NBSpliceRes: [GetDSGenes](#), [GetDSResults](#), [GetGeneResults](#), [NBSpliceRes-class](#), [NBSpliceRes](#), [myDSResults](#), [plotGeneResults](#), [plotRatiosDisp](#), [plotVolcano](#)

## Examples

```
data(myDSResults, package="NBSplice")
myResults<-results(myDSResults, filter=FALSE)
myLowExpIdx<-lowExpIndex(myDSResults)
myContrast<-contrast(myDSResults)

myNewDSResults<-NBSpliceRes(myResults, myLowExpIdx, myContrast)
```

---

NBSpliceRes-print            *Print an NBSpliceRes object.*

---

### Description

Generic `print` method for NBSpliceRes class and descendants.

### Usage

```
## S4 method for signature 'NBSpliceRes'
print(x, ...)
```

### Arguments

| | |
|---|---|
| x | NBSpliceRes class object. |
| ... | Included for generic print compatibility. |

### Value

Console output of the object.

### Note

see full example in `NBSpliceRes-class`

### Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com>, and Elmer A. Fernandez <efernandez@bdmg.com.ar>

### Examples

```
data(myDSResults, package="NBSplice")

print(myDSResults)
```

---

NBSpliceRes-show          *Show method for the NBSpliceRes class.*

---

### Description

show an NBSpliceRes object

### Usage

```
## S4 method for signature 'NBSpliceRes'
show(object)
```

### Arguments

object          NBSpliceRes class object

### Value

Console output of the object

### Note

see full example in [NBSpliceRes-class](NBSpliceRes-class)

### Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

### Examples

```
data(myDSResults, package="NBSplice")

show(myDSResults)
```

---

NBTest                    *Differential splicing analysis.*

---

### Description

NBTest is the main function of NBSplice. It fits the negative binomial models for all genes and performs the corresponding hypothesis tests to evaluate the occurrence of differential splicing.

## Usage

```
NBTest(object, colName = "condition", test = c("F", "Chisq"),
    contrast = c(levels(expData(object)[,colName])[seq_len(2)]),
    BPPARAM = bpparam())

## S4 method for signature 'IsoDataSet'
NBTest(object, colName = "condition", test = c("F",
    "Chisq"), contrast = c(levels(expData(object)[,colName])[seq_len(2)]),
    BPPARAM = bpparam())
```

## Arguments

| | |
|---|---|
| object | IsoDataSet class object. |
| colName | Character indicating the name of the column in the design matrix to be considered for mean expression calculations per experimental condition. |
| test | Character indicating the name of the distribution to assume for linear hypothesis statistic. Could be "F" or "chisq". |
| contrast | Character vector with the names of the two levels of the experimental factor to be contrasted. |
| BPPARAM | An optional BiocParallelParam instance defining the parallel back-end to be used during evaluation. |

## Value

A NBSpliceRes object with the obtained results.

## Note

see full example in [IsoDataSet-class](IsoDataSet-class)

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

[IsoDataSet](IsoDataSet)

Other IsoDataSet: [IsoDataSet-class](IsoDataSet-class), [IsoDataSet](IsoDataSet), [buildData](buildData), [buildLowExpIdx](buildLowExpIdx), [designMatrix](designMatrix), [geneIso](geneIso), [initialize](initialize), [isoCountsData](isoCountsData), [myIsoDataSet](myIsoDataSet)

## Examples

```
## Data loading
data(myIsoDataSet, package="NBSplice")

## Identifying Low expressed Isoforms
myIsoDataSet<-buildLowExpIdx(myIsoDataSet)
```

```
##Arguments definition
colName<-"condition"
test<-"F"

## Differential splicing test
myDSResults<-NBTest(myIsoDataSet, colName, test)
```

---

plotGeneResults          *Method to obtain isoform's relative expression barplot for an specific*
                         *gene.*

---

### Description

`plotGeneResults` returns ggplot object which illustrates the isoform's relative expression in the
two contrasted conditions.

### Usage

```
plotGeneResults(myNBRes, gene, filterLowExpIso = TRUE,
    filterNotSignificant = TRUE, adjusted = TRUE, p.value = 0.05,
    group = TRUE)

## S4 method for signature 'NBSpliceRes'
plotGeneResults(myNBRes, gene, filterLowExpIso = TRUE,
    filterNotSignificant = TRUE, adjusted = TRUE, p.value = 0.05,
    group = TRUE)
```

### Arguments

| | |
|---|---|
| myNBRes | NBSpliceRes class object. |
| gene | Character indicating the gene name. |
| filterLowExpIso | |
| | Logical indicating if lower-expression isoforms should be filtered out. |
| filterNotSignificant | |
| | Logical indicating if not significant isoforms should be filtered out. |
| adjusted | Logical indicating if adjusted p values should be used. |
| p.value | Numeric value between 0 and 1 giving the required family-wise error rate or false discovery rate. |
| group | Logical indicating if isoforms bars should be stacked or not |

### Value

A ggplot object.

## Note

see full example in `NBSpliceRes-class`

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

`NBSpliceRes`

Other NBSpliceRes: `GetDSGenes`, `GetDSResults`, `GetGeneResults`, `NBSpliceRes-class`, `NBSpliceRes-initialize`, `NBSpliceRes`, `myDSResults`, `plotRatiosDisp`, `plotVolcano`

## Examples

```
data(myDSResults, package="NBSplice")
gene<-results(myDSResults)[,"gene"][1]
##Plot gene results filtering low expressed isoforms
g<-plotGeneResults(myDSResults, gene)
if(interactive()){
g
}
##Plot gene results keeping low expressed isoforms
g<-plotGeneResults(myDSResults, gene, filterLowExpIso=FALSE)
if(interactive()){
g
}
##Plot isoform bar plots keeping low expressed isoforms
g<-plotGeneResults(myDSResults, gene, filterLowExpIso=FALSE, group=FALSE)
if(interactive()){
g
}
```

---

plotRatiosDisp        *Plot the dispersion of isoform's relative expression for significant genes.*

---

## Description

`plotRatiosDisp` returns a ggplot object with the scatter plot of isoform's relative expression in the contrasted conditions. Isoforms are colored according to the differential splicing status of the gene where they come from.

## Usage

```
plotRatiosDisp(myNBRes, adjusted = TRUE, p.value = 0.05)

## S4 method for signature 'NBSpliceRes'
plotRatiosDisp(myNBRes, adjusted = TRUE,
    p.value = 0.05)
```

## Arguments

| | |
|---|---|
| myNBRes | NBSpliceRes class object. |
| adjusted | Logical indicating if adjusted p values should be used. |
| p.value | Numeric value between 0 and 1 giving the required family-wise error rate or false discovery rate. |

## Value

A ggplot object.

## Note

see full example in [NBSpliceRes-class](#)

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

[NBSpliceRes](#)

Other NBSpliceRes: [GetDSGenes](#), [GetDSResults](#), [GetGeneResults](#), [NBSpliceRes-class](#), [NBSpliceRes-initialize](#), [NBSpliceRes](#), [myDSResults](#), [plotGeneResults](#), [plotVolcano](#)

## Examples

```
data(myDSResults, package="NBSplice")

g<-plotRatiosDisp(myDSResults)
if(interactive()){
g
}
```

---

plotVolcano                      *Method to obtain the Volcano Plot.*

---

## Description

`plotVolcano` returns a generalized volcano plot where the x-axis represents the difference between isoform's relative expression in the contrasted conditions. Isoforms are colored according to the differential splicing status of the gene where they come from.

## Usage

```
plotVolcano(myNBRes, adjusted = TRUE, p.value = 0.05)

## S4 method for signature 'NBSpliceRes'
plotVolcano(myNBRes, adjusted = TRUE,
    p.value = 0.05)
```

## Arguments

| | |
|---|---|
| myNBRes | NBSpliceRes class object. |
| adjusted | Logical indicating if adjusted p values should be used. |
| p.value | Numeric value between 0 and 1 giving the required family-wise error rate or false discovery rate. |

## Value

A ggplot object.

## Note

see full example in [NBSpliceRes-class](#)

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## See Also

[NBSpliceRes](#)

Other NBSpliceRes: [GetDSGenes](#), [GetDSResults](#), [GetGeneResults](#), [NBSpliceRes-class](#), [NBSpliceRes-initialize](#), [NBSpliceRes](#), [myDSResults](#), [plotGeneResults](#), [plotRatiosDisp](#)

## Examples

```
data(myDSResults, package="NBSplice")

g<-plotVolcano(myDSResults)
if(interactive()){
g
}
```

---

print                         *Print an IsoDataSet object.*

---

## Description

Generic print method for IsoDataSet class and descendants.

## Usage

```
## S4 method for signature 'IsoDataSet'
print(x, ...)
```

## Arguments

| | |
|---|---|
| x | IsoDataSet class object. |
| ... | Included for generic print compatibility. |

## Value

Console output of the object.

## Note

see full example in [IsoDataSet-class](IsoDataSet-class)

## Author(s)

Gabriela A. Merino <merino.gabriela33@gmail.com>, and Elmer A. Fernandez <efernandez@bdmg.com.ar>

## Examples

```
## Data loading
data(myIsoDataSet, package="NBSplice")

print(myIsoDataSet)
```

---

show                    *Show method for the IsoDataSet class.*

---

## Description

show an IsoDataSet object

## Usage

```
## S4 method for signature 'IsoDataSet'
show(object)
```

## Arguments

| | |
|---|---|
| object | IsoDataSet class object |

## Value

Console output of the object

## Note

see full example in [IsoDataSet-class](IsoDataSet-class)

**Author(s)**

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

**Examples**

```
## Data loading
data(myIsoDataSet, package="NBSplice")

show(myIsoDataSet)
```

---

  totalGeneCounts          *Auxiliary function to build gene expression matrix*

---

**Description**

totalGeneCounts obtains the total counts for a gene in a sample by the sum of the counts of all its isoforms. This count is performed before any filter step to avoid erroneus estimation of isoform ratios.

**Usage**

```
totalGeneCounts(isoCounts, geneIso, BPPARAM = bpparam())
```

**Arguments**

| | |
|---|---|
| isoCounts | Matrix containing the isoform expression values with isoforms in rows and samples in columns. Object row names should be the names of the isoforms and the column names, the names of each sample. |
| geneIso | Data.frame with two columns called "isoform_id" and "gene_id" especifying which are the isoform (rows of the expression matrix) for each gene |
| BPPARAM | A bpparam object specifying the number of cpus to be used. |

**Value**

A matrix of the same size as the isoCounts matrix with the total counts for each gene in each sample in CPM scale.

**Author(s)**

Gabriela A. Merino <merino.gabriela33@gmail.com> and Elmer A. Fernandez <efernandez@bdmg.com.ar>

**Examples**

```
data(isoCounts, package="NBSplice")
data(geneIso, package="NBSplice")

myGeneCounts<-totalGeneCounts(isoCounts, geneIso)
```

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