

# Package ‘Polyfit’

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**Type** Package

**Title** Add-on to DESeq to improve p-values and q-values

**Version** 1.14.0

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**biocViews** DifferentialExpression, Sequencing, RNASeq, GeneExpression

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**Depends** DESeq

**Suggests** BiocStyle

**Description** Polyfit is an add-on to the packages DESeq which ensures the p-value distribution is uniform over the interval [0, 1] for data satisfying the null hypothesis of no differential expression, and uses an adapted Storey-Tibshiran method to calculate q-values.

**License** GPL (>= 3)

**git\_url** <https://git.bioconductor.org/packages/Polyfit>

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Polyfit-package

*Polyfit add-on to DESeq*

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## Description

implementation the Polyfit add-on to DESeq described in the paper "Improved error estimates for the analysis of differential expression from RNA-seq data"

## Details

Package: Polyfit  
Type: Package  
Version: 0.99.3  
Date: 2014-08-06  
License: GPL(>=3)

Polyfit is an add-on to the negative-binomial based packages DESeq for two-class detection of differential expression which ensures the p-value distribution is uniform over the interval [0, 1] for data satisfying the null hypothesis of no differential expression. The first component is the function `pfNbinomTest` which replaces the function `nbinomTest` in DESeq. Its purpose is to smooth point singularities, particularly one at  $p = 1$ , in the p-value distribution caused by calculating calculating p-values from a discrete distribution. The output from this function should then be passed to the second component, the function `link{levelPValues}`. Its purpose is to apply a variant of the Storey-Tibshirani procedure to shift the p-values so that those corresponding to the null hypothesis have a uniform distribution, and to calculate corresponding q-values (or 'adjusted p-values') for controlling errors via the false discovery rate.

## Author(s)

Conrad Burden

Maintainer: conrad.burden@anu.edu.au

## References

Burden, C.J., Qureshi, S. and Wilson, S.R. (2014). *Error estimates for the analysis of differential expression from RNA-seq count data*, PeerJ PrePrints 2:e400v1.

Robinson, M., McCarthy, D., and Smyth, G. (2010). *edgeR: a Bioconductor package for differential expression analysis of digital gene expression data*. *Bioinformatics*, **26**, 139-140.

Anders, S. and Huber, W. (2010). *Differential expression analysis for sequence count data*. *Genome Biology*, **11**(10), R106.

## Examples

```
# Example using DESeq
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateDispersions( cds )
nbTPolyfit <- pfNbinomTest( cds, "A", "B" )
```

```
lP <- levelPValues(nbTPolyfit$pval)
pvalTab <- cbind(origPval=nbTPolyfit$pval, correctedPval=lP$pValueCorr, qval=lP$qValueCorr)
cat("\n Original and corrected P-values from DESeq \n")
head(pvalTab)
```

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levelPValues	<i>Level P-values</i>
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### Description

Function to level out a P-value spectrum generated by the Polyfit extension of DESeq by fitting a quadratic function to the right hand portion of the spectrum, produce 'corrected' p-values and q-values using an adapted version of the Storey-Tibsharini procedure

### Usage

```
levelPValues(oldPvals, plot = FALSE)
```

### Arguments

oldPvals	an array of p-values produced by the Polyfit replacement of the DESeq function <code>pfNbinomTest()</code> or the Plyfit replacement of the edgeR function <code>pfExactTest()</code>
plot	TRUE to plot original and corrected pvalue spectra; FALSE not to plot

### Details

`levelPValues` should only be used with P-values generated by the Polyfit function `pfNbinomTest`, and not with P-values generated by `nbinomTest`.

### Value

List containing

<code>pi0estimate</code>	an estimate of the proportion of genes not differentially expressed
<code>lambdaOptimal</code>	the point in the p-value spectrum past which a quadratic is fitted
<code>pValueCorr</code>	p-values calculated from the levelled spectrum
<code>qValueCorr</code>	q-values calculated from the levelled spectrum
<code>qValueCorrBH</code>	q-values calculated from <code>pValueCorr</code> using Benjamini-Hochberg

### Author(s)

Conrad Burden

### References

Burden, C.J., Qureshi, S. and Wilson, S.R. (2014). *Error estimates for the analysis of differential expression from RNA-seq count data*, PeerJ PrePrints 2:e400v1.

**Examples**

```

cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateDispersions( cds )
nbTPolyfit <- pfNbinomTest( cds, "A", "B" )
lP <- levelPValues(nbTPolyfit$pval, plot=TRUE)
pvalTab <- cbind(origPval=nbTPolyfit$pval, correctedPval=lP$pValueCorr)
cat("\n Original and corrected P-values from DESeq \n")
head(pvalTab)

```

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pfNbinomTest	<i>The Polyfit extension to the DESeq functions nbinomTest() and nbinomTestForMatrices()</i>
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**Description**

Polyfit extensions to the DESeq functions [nbinomTest](#) and [nbinomTestForMatrices](#) which test for differences between the base means of two conditions (i.e., for differential expression in the case of RNA-Seq).

**Usage**

```

pfNbinomTest(cds, condA, condB, pvals_only = FALSE, eps = NULL)
pfNbinomTestForMatrices(countsA, countsB, sizeFactorsA, sizeFactorsB, dispsA, dispsB )

```

**Arguments**

cds	a CountDataSet with size factors and raw variance functions
condA	one of the conditions in 'cds'
condB	another one of the conditions in 'cds'
pvals_only	return only a vector of (unadjusted) p values instead of the data frame described below
eps	This argument is no longer used. Do not use it
countsA	A matrix of counts, where each column is a replicate
countsB	Another matrix of counts, where each column is a replicate
sizeFactorsA	Size factors for the columns of the matrix 'countsA'
sizeFactorsB	Size factors for the columns of the matrix 'countsB'
dispsA	The dispersions for 'countsA', a vector with one value per gene
dispsB	The same for 'countsB'

**Details**

These functions have the same behaviour as the DESeq functions [nbinomTest](#) and [nbinomTestForMatrices](#), except that the 'flagpole' in the P-value histogram, particularly at  $p = 1$  is redistributed using the function [twoSidedPValueFromDiscrete](#).

**Value**

pfNbinomTest gives a data frame with the following columns:

id	The ID of the observable, taken from the row names of the counts slots.
baseMean	The base mean (i.e., mean of the counts divided by the size factors) for the counts for both conditions
baseMeanA	The base mean (i.e., mean of the counts divided by the size factors) for the counts for condition A
baseMeanB	The base mean for condition B
foldChange	The ratio meanB/meanA
log2FoldChange	The log2 of the fold change
pval	The p value for rejecting the null hypothesis 'meanA==meanB'
padj	The adjusted p values (adjusted with 'p.adjust( pval, method="BH")')

pfNbinomTestForMatrices gives a vector of unadjusted p values, one for each row in the counts matrices.

**Author(s)**

Conrad Burden, conrad.burden@anu.edu.au, based on software by Simon Anders

**References**

Burden, C.J., Qureshi, S. and Wilson, S.R. (2014). *Error estimates for the analysis of differential expression from RNA-seq count data*, PeerJ PrePrints 2:e400v1.

Anders, S. and Huber, W. (2010). *Differential expression analysis for sequence count data*. Genome Biology, **11**(10), R106.

**Examples**

```

cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateDispersions( cds )
nbT <- nbinomTest( cds, "A", "B" )
head( nbT )
nbTPolyfit <- pfNbinomTest( cds, "A", "B" )
head( nbTPolyfit )

oldpar <- par(mfrow=c(1,2))
hist(nbT$pval,breaks=seq(0,1,by=0.01),
     xlab="P-value", main="DESeq")
hist(nbTPolyfit$pval,breaks=seq(0,1,by=0.01),
     xlab="P-value", main="polyfit-DESeq")
par(oldpar)

```

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`twoSidedPValueFromDiscrete`*Two sided P-value from discrete distribution*

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**Description**

Function to calculate a 2-sided p-value of an observation *xobs* for a finite discrete distribution

$$Prob(X = xobs) = probs[xobs + 1]$$

over the range *xobs* in  $(0, 1, \dots, xmax)$  by "squaring off" the distribution to a continuous distribution

**Usage**

```
twoSidedPValueFromDiscrete(probs, xobs)
```

**Arguments**

<code>probs</code>	an array containing the probabilities that $X$ takes the values $0, 1, \dots, xmax$
<code>xobs</code>	a single observed value of $X$

**Details**

Note that the returned 2-sided p-value contains a random component, i.e. a given set of input parameters returns a different result each run

**Value**

A real valued randomised p-value between 0 and 1. If *xobs* is generated with randomly with probability  $probs[xobs + 1]$  the returned value will be uniformly distributed on the interval  $[0, 1]$ .

**Author(s)**

Conrad Burden

**Examples**

```
pr <- dbinom(0:5, size=5, prob=0.4)
xSample <- rbinom(10000, size=5, prob=0.4)
pvalues <- c()
for(x in xSample){
  pvalues <- c(pvalues, twoSidedPValueFromDiscrete(pr, x))
}
hist(pvalues)
```

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