Package 'ROSeq'

November 12, 2026

Type Package

Title Modeling expression ranks for noise-tolerant differential expression analysis of scRNA-Seq data

Version 1.24.0

Description ROSeq - A rank based approach to modeling gene expression with filtered and normalized read count matrix. ROSeq takes filtered and normalized read matrix and cell-annotation/condition as input and determines the differentially expressed genes between the contrasting groups of single cells. One of the input parameters is the number of cores to be used.

URL https://github.com/krishan57gupta/ROSeq

BugReports https://github.com/krishan57gupta/ROSeq/issues

License GPL-3

Encoding UTF-8

LazyData true

RoxygenNote 7.1.1

Depends R (>= 4.0)

biocViews GeneExpression, DifferentialExpression, SingleCell

Imports pbmcapply, edgeR, limma

Suggests knitr, rmarkdown, testthat, RUnit, BiocGenerics

VignetteBuilder knitr

git_url https://git.bioconductor.org/packages/ROSeq

git_branch RELEASE_3_23

git_last_commit 3437ff3

git_last_commit_date 2026-04-28

Repository Bioconductor 3.23

Date/Publication 2026-11-11

Author Krishan Gupta [aut, cre],
Manan Lalit [aut],
Aditya Biswas [aut],
Abhik Ghosh [aut],
Debarka Sengupta [aut]

Maintainer Krishan Gupta <krishang@iiitd.ac.in>

Contents

computeDEG 2
findParams 3
getd 4
getDataStatistics 4
getdu1da 5
getdu1db 5
getdu2da 6
getdu2db 6
getdvda 7
getdvdb 7
getI 8
getu1 8
getu2 9
getv 9
initiateAnalysis 10
L_Tung_single 10
minimizeNLL 11
ROSeq 12
TMMnormalization 13

Index **14**

`computeDEG` *Computes differential expression for the gene in question, by comparing the optimal parameters for sub-populations one and two*

Description

Uses the (asymptotically) optimum two-sample Wald test based on the MLE of the parameters and its asymptotic variances given by the inverse of the Fisher information matrix

Usage

```
computeDEG(results_1, results_2)
```

Arguments

results_1	A vector corresponding to sub-population one and containing 5 values (a, b, A, number of bins, R2)
results_2	A vector corresponding to sub-population two and containing 5 values (a, b, A, number of bins, R2)

Value

T The Wald test statistic for testing the null hypothesis

See Also

getI, findParams

---

findParams *Finds the optimal values of parameters a and b that model the probability distribution of ranks, by Maximising the Log-Likelihood*

Description

Takes in as input the read count data corresponding to one sub-population and the typical gene statistics. Then it splits the entire range into equally sized bins of size $k * \sigma$, where k is a scalar with a default value of 0.05, and σ is the standard deviation of the pulled expression estimates across the cell-groups. Each of these bins corresponds to a rank. Therefore, for each group, cell frequency for each bin maps to a rank. These frequencies are normalized group-wise by dividing by the total cell count within a concerned group.

Usage

```
findParams(ds, geneStats)
```

Arguments

ds	The (normalized and filtered) read count data corresponding to a sub-population
geneStats	A vector containing 7 values corresponding to the gene data (maximum, minimum, mean, standard deviation, upper multiple of the standard deviation, lower multiple of standard deviation and log_2(fold change))

Value

results A vector containing 5 values (a, b, A, number of bins, R2)

getd *Finds the double derivative of A*

Description

Finds the double derivative of A with with respect to a, (a, b), b , (a, b) in respective templates from right to left. This first derivative is evaluated at the optimal (a_hat, b_hat). u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getd(u1, v, du1da, dvda)
```

Arguments

u1	u1
v	v
du1da	First derivative of u1 with respect to parameter a
dvda	First derivative of v with respect to parameter a

Value

d2logAda2

getDataStatistics *Evaluates statistics of the read counts corresponding to the gene*

Description

Takes in the complete read count vector corresponding to the gene (sp) and also the data corresponding to the two sub-populations (sp1 and sp2)

Usage

```
getDataStatistics(sp, spOne, spTwo)
```

Arguments

sp	The complete (normalized and filtered) read count data corresponding to the gene in question
spOne	The (normalized and filtered) read count data corresponding to the first sub-population
spTwo	The (normalized and filtered) read count data corresponding to the second sub-population

Value

geneStats A vector containing 6 values corresponding to the gene data(maximum, minimum, mean, standard deviation, upper multiple of standard deviation and lower multiple of standard deviation)

`getdu1da` *Finds the first derivative of u1 with respect to a. This first derivative is evaluated at the optimal (a_hat, b_hat).*

Description

u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getdu1da(coefficients, r)
```

Arguments

`coefficients`	the optimal values of a and b
`r`	the rank vector

Value

du1da

`getdu1db` *Finds the first derivative of u1 with respect to b. This first derivative is evaluated at the optimal (a_hat, b_hat).*

Description

u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getdu1db(coefficients, r)
```

Arguments

`coefficients`	the optimal values of a and b
`r`	the rank vector

Value

du1db

`getdu2da` *Finds the first derivative of u2 with respect to a. This first derivative is evaluated at the optimal (a_hat, b_hat).*

Description

u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getdu2da(coefficients, r)
```

Arguments

`coefficients`	the optimal values of a and b
`r`	the rank vector

Value

du2da

`getdu2db` *Finds the first derivative of u2 with respect to b. This first derivative is evaluated at the optimal (a_hat, b_hat).*

Description

u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getdu2db(coefficients, r)
```

Arguments

`coefficients`	the optimal values of a and b
`r`	the rank vector

Value

du2db

`getdvda` *Finds the first derivative of v with respect to a. This first derivative is evaluated at the optimal (a_hat, b_hat).*

Description

u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getdvda(coefficients, r)
```

Arguments

`coefficients`	the optimal values of a and b
`r`	the rank vector

Value

dvda

`getdvdb` *Finds the first derivative of v with respect to b. This first derivative is evaluated at the optimal (a_hat, b_hat).*

Description

u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getdvdb(coefficients, r)
```

Arguments

`coefficients`	the optimal values of a and b
`r`	the rank vector

Value

dvdb

getI *Computes the Fisher Information Matrix*

Description

The Fisher Information Matrix and its derivatives are essential to evulate the minima of log likelihood

Usage

```
getI(results)
```

Arguments

results	A vector containing 5 values (a, b, A, number of bins, R2)

Value

I The Fisher Information Matrix

getu1 *Computes u1*

Description

u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getu1(coefficients, r)
```

Arguments

coefficients	the optimal values of a and b
r	the rank vector

Value

u1

getu2 *Computes u2*

Description

u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getu2(coefficients, r)
```

Arguments

coefficients	the optimal values of a and b
r	the rank vector

Value

u2

getv *Computes v*

Description

u1, v and u2 constitute the equations required for evaluating the first and second order derivatives of A with respect to parameters a and b

Usage

```
getv(coefficients, r)
```

Arguments

coefficients	the optimal values of a and b
r	the rank vector

Value

v

initiateAnalysis *Computes differential analysis for a given gene*

Description

Takes in the row index which corresponds to a gene and evaluates for differential expression across two cell types.

Usage

```
initiateAnalysis(gene, scdata, scgroups, classOne, classTwo)
```

Arguments

gene	The row index of the normalised and filtered, read count matrix
scdata	The normalised and filtered, read count matrix
scgroups	The location of the two sub-populations
classOne	The location of the first sub-population, for example, sample names as given as columns names
classTwo	The location of thesecond sub-population, for example, sample names as given as columns names

Value

combinedResults A vector containing 12 values (gr1: a, g1: b, gr1: A, gr1: number of bins, gr1: R2, gr2: a, gr2: b, gr2: A, gr2: number of bins, gr2: R2, T, p)

L_Tung_single *Single cell samples for DE genes analysis*

Description

Three replicates from three human induced pluripotent stem cell (iPSC) lines were considered. 96 single cells were considered in each of the three replicates corresponding to one of the three individuals (these shall be referred to by their labels NA19098,NA19101 and NA19239)

Usage

```
data("L_Tung_single")
```

Format

The format is: list of cells corresponding NA19098 versus NA19101 and groups labels.

Details

filtered and normalized data

Source

Tung, P.-Y.et al.Batch effects and the effective design of single-cell geneexpression studies.Scientific reports7, 39921 (2017).

References

Tung, P.-Y.et al.Batch effects and the effective design of single-cell geneexpression studies.Scientific reports7, 39921 (2017).

Examples

```
data(L_Tung_single)
## summary(ROSeq::L_Tung_single)
```

`minimizeNLL` *Minimizes the Negative Log-Likelihood by iterating across values of parameters a and b*

Description

Takes in as input a vector of values (coefficients), the number of bins and the normalized probability dsitribution of ranks

Usage

```
minimizeNLL(coefficients, r, readCount)
```

Arguments

`coefficients`	A vector containing two values for a and b
`r`	The number of bins
`readCount`	A vector of (normalized) frequency of read counts that fall within each bin

Value

NLL Negative-Log Likelihood for the input coefficients

See Also

`findParams`

ROSeq *Modeling expression ranks for noise-tolerant differential expression analysis of scRNA-Seq data*

Description

Takes in the complete filtered and normalized read count matrix, the location of the two sub-populations and the number of cores to be used

Usage

```
ROSeq(countData, condition, numCores = 1)
```

Arguments

countData	The normalised and filtered, read count matrix, with row names as genes name/ID and column names as sample id/name
condition	Labels for the two sub-populations
numCores	The number of cores to be used

Value

pValues and FDR adjusted p significance values

Examples

```
countData<-list()
countData$count<-ROSeq::L_Tung_single$NA19098_NA19101_count
countData$group<-ROSeq::L_Tung_single$NA19098_NA19101_group
head(countData$count)
gene_names<-rownames(countData$count)
countData$count<-apply(countData$count,2,function(x) as.numeric(x))
rownames(countData$count)<-gene_names
countData$count<-countData$count[,colSums(countData$count> 0) > 2000]
g_keep <- apply(countData$count,1,function(x) sum(x>2)>=3)
countData$count<-countData$count[g_keep,]
countData$count<-limma::voom(ROSeq::TMMnormalization(countData$count))
output<-ROSeq(countData=countData$count$E, condition = countData$group)
output
```

TMMnormalization *TMM Normalization.*

Description

Trimmed Means of M values (TMM) normalization (on the basis of edgeR package)

Usage

```
TMMnormalization(countTable)
```

Arguments

countTable	The filtered, read count matrix, with row names as genes name/ID and column names as sample id/name

Value

countTableTMM

Examples

```
countData<-list()
countData$count<-ROSeq::L_Tung_single$NA19098_NA19101_count
countData$group<-ROSeq::L_Tung_single$NA19098_NA19101_group
head(countData$count)
gene_names<-rownames(countData$count)
countData$count<-apply(countData$count,2,function(x) as.numeric(x))
rownames(countData$count)<-gene_names
countData$count<-countData$count[,colSums(countData$count> 0) > 2000]
g_keep <- apply(countData$count,1,function(x) sum(x>2)>=3)
countData$count<-countData$count[g_keep,]
countTableTMM<-ROSeq::TMMnormalization(countData$count)
countTableTMM
```

Index

∗ **datasets**
L_Tung_single, 10

computeDEG, 2

findParams, *3*, 3, *11*

getd, 4
getDataStatistics, 4
getdu1da, 5
getdu1db, 5
getdu2da, 6
getdu2db, 6
getdvda, 7
getdvdb, 7
getI, *3*, 8
getu1, 8
getu2, 9
getv, 9

initiateAnalysis, 10

L_Tung_single, 10

minimizeNLL, 11

ROSeq, 12

TMMnormalization, 13