Package ‘ROSeq’

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Type Package

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

Version 1.16.0

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, markdown, testthat, RUnit, BiocGenerics

VignetteBuilder knitr

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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)
findParams

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

gEI, 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 \( k \times \sigma \), where \( k \) is a scalar with a default value of 0.05, and \( \sigma \) 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 respect to $a, (a, b), b, (a, b)$ in respective templates from right to left. This first derivative is evaluated at the optimal $(\hat{a}, \hat{b})$. $u_1$, $v$ and $u_2$ constitute the equations required for evaluating the first and second order derivatives of $A$ with respect to parameters $a$ and $b$.

Usage
getd($u_1$, $v$, $du_1da$, $dvda$)

Arguments
- $u_1$: First derivative of $u_1$ with respect to parameter $a$
- $v$: First derivative of $v$ with respect to parameter $a$
- $du_1da$: $u_1$
- $dvda$: $v$

Value
d$2\log_{\hat{a}}a^2$

gerDataStatistics

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
gerDataStatistics($sp$, $spOne$, $spTwo$)

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

getu2da(coefficients, r)

Arguments

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

Value

du2da

getu2db

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

getu2db(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 evaluate 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
\[ \text{initiateAnalysis}(\text{gene}, \text{scdata}, \text{scgroups}, \text{classOne}, \text{classTwo}) \]

Arguments
- \text{gene}: The row index of the normalised and filtered, read count matrix
- \text{scdata}: The normalised and filtered, read count matrix
- \text{scgroups}: The location of the two sub-populations
- \text{classOne}: The location of the first sub-population, for example, sample names as given as columns names
- \text{classTwo}: The location of the second sub-population, for example, sample names as given as columns names

Value
\text{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
\[ \text{data}(\text{"L_Tung\_single"}) \]

Format
The format is: list of cells corresponding NA19098 versus NA19101 and groups labels.
Details
filtered and normalized data

Source

References

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