

# Package ‘srnadiff’

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**Type** Package

**Title** Differential Expression of Small RNA-Seq

**Version** 1.4.0

**Maintainer** Matthias Zytnicki <matthias.zytnicki@inra.fr>

**Description** Differential expression of small RNA-seq  
when reference annotation is not given.

**License** GPL-3

**Encoding** UTF-8

**LazyData** true

**Depends** R (>= 3.5)

**SystemRequirements** C++11

**Imports** Rcpp (>= 0.12.8), methods, utils, devtools, BiocStyle,  
S4Vectors, GenomeInfoDb, rtracklayer, SummarizedExperiment,  
IRanges, GenomicRanges, DESeq2, Rsamtools, GenomicFeatures,  
GenomicAlignments, ggplot2, BiocParallel

**LinkingTo** Rcpp

**RoxygenNote** 6.0.1

**biocViews** ImmunoOncology, GeneExpression, Coverage, SmallRNA,  
Epigenetics, StatisticalMethod, Preprocessing,  
DifferentialExpression

**Suggests** knitr, rmarkdown, testthat

**VignetteBuilder** knitr

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**Author** Zytnicki Matthias [aut, cre]

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---

buildDataHmm	<i>Read the coverage and extract expressed regions</i>
--------------	--

---

**Description**

Read the coverage and extract expressed regions

**Usage**

```
buildDataHmm(object)
```

**Arguments**

object            An srnadiff object.

**Value**

The selected values: a list of vectors of integers.

---

computePvalues	<i>Compute p-values of the selected counts.</i>
----------------	---

---

**Description**

Compute p-values of the selected counts.

**Usage**

```
computePvalues(object, counts)
```

**Arguments**

object            An srnadiff object.  
 counts            The counts: a list of vectors or integers.

**Value**

The p-values: a list of numeric.

---

plotRegion	<i>Plot a region</i>
------------	----------------------

---

**Description**

Plot a region

**Usage**

```
plotRegion(object, region)
```

**Arguments**

object            An srnadiff object.  
 region            A GenomicRange object.

**Value**

A ggplot object.

**Examples**

```
exp <- sRNADiffExample()
exp <- runAll(exp)
plotRegion(exp, regions(exp, 0.05)[1])
```

---

rcpp_buildHmm	<i>Compute unique counts.</i>
---------------	-------------------------------

---

**Description**

Compute unique counts.

**Usage**

```
rcpp_buildHmm(lengths, values, chromosomeSizes, minDepth)
```

**Arguments**

lengths	the sizes of the RLEs (one list per chromosome)
values	the values of the RLEs (one list per chromosome)
chromosomeSizes	the sizes of the chromosomes
minDepth	the minimum read coverage

**Value**

the unique counts

---

rcpp_naive	<i>Compute naive method.</i>
------------	------------------------------

---

**Description**

Compute naive method.

**Usage**

```
rcpp_naive(lengths, values, chromosomeSizes, depth, distance, size)
```

**Arguments**

lengths	the sizes of the RLEs (one list per chromosome)
values	the values of the RLEs (one list per chromosome)
chromosomeSizes	the sizes of the chromosomes
depth	minimum number of reads per position
distance	threshold to merge consecutive regions
size	minimum region size

**Value**

the unique counts

---

rcpp_normalization	<i>Normalize counts (and changes the input values)</i>
--------------------	--

---

**Description**

Normalize counts (and changes the input values)

**Usage**

```
rcpp_normalization(lengths, values, chromosomeSizes, librarySizes)
```

**Arguments**

lengths	the sizes of the RLEs (one list per chromosome)
values	the values of the RLEs (one list per chromosome)
chromosomeSizes	the sizes of the chromosomes
librarySizes	number of elements per sample

**Value**

nothing (but transform the values instead)

---

rcpp_slice	<i>Compute unique counts.</i>
------------	-------------------------------

---

**Description**

Compute unique counts.

**Usage**

```
rcpp_slice(lengths, values, chromosomeSizes, minDepth, minSize, maxSize,
            minDifference)
```

**Arguments**

lengths	the sizes of the RLEs (one list per chromosome)
values	the values of the RLEs (one list per chromosome)
chromosomeSizes	the sizes of the chromosomes
minDepth	minimum coverage
minSize	minimum region size
maxSize	maximum region size
minDifference	minimum difference between 2 regions

**Value**

selected regions

---

rcpp_viterbi	<i>Run the Viterbi algorithm on the HMM.</i>
--------------	--

---

**Description**

Run the Viterbi algorithm on the HMM.

**Usage**

```
rcpp_viterbi(chromosomeSizes, transitions, emissions, emissionThreshold, starts,
             counts, pvalues, lengths, values, minDepth, minSize, maxSize)
```

**Arguments**

chromosomeSizes	the sizes of the chromosomes
transitions	the transition log-probabilities
emissions	the emission log-probabilities
emissionThreshold	the emission threshold
starts	the start log-probabilities
counts	the unique counts
pvalues	the p-values of the counts
lengths	the sizes of the RLEs (one list per chromosome)
values	the values of the RLEs (one list per chromosome)
minDepth	the minimum read coverage
minSize	the minimum size region
maxSize	the maximum size region

**Value**

a segmentation of the chromosomes

---

readAnnotation	<i>Segmentation using an annotation file.</i>
----------------	---

---

**Description**

Segmentation using an annotation file.

**Usage**

```
readAnnotation(fileName, source = NULL, feature = NULL, name = NULL)
```

**Arguments**

fileName	The annotation file name in GFF/GTF format.
source	If not NULL, only lines with this source (2nd field) are imported.
feature	If not NULL, only lines with this feature (3rd field) are imported.
name	If not NULL, use this tag as annotation name.

**Value**

A GRanges.

**Examples**

```
dir      <- system.file("extdata", package="srnadiff", mustWork = TRUE)
gtffFile <- file.path(dir, "Homo_sapiens.GRCh38.76.gtf.gz")
annotation <- readAnnotation(gtffFile, source="miRNA", feature="gene",
                             name="gene_name")
```

---

readMiRBaseMatureAnnotation

*Segmentation using an miRBase annotation file and use mature miRNAs.*

---

**Description**

Segmentation using an miRBase annotation file and use mature miRNAs.

**Usage**

```
readMiRBaseMatureAnnotation(fileName)
```

**Arguments**

fileName	The annotation file name in GFF/GTF format.
----------	---

**Value**

A GRanges.

**Examples**

```
dir      <- system.file("extdata", package="srnadiff", mustWork = TRUE)
gfffFile <- file.path(dir, "mirbase21_GRCh38.gff3")
annotation <- readMiRBaseMatureAnnotation(gfffFile)
```

---

readMiRBasePreAnnotation

*Segmentation using an miRBase annotation file and use precursor miRNAs.*

---

### Description

Segmentation using an miRBase annotation file and use precursor miRNAs.

### Usage

```
readMiRBasePreAnnotation(fileName)
```

### Arguments

fileName            The annotation file name in GFF/GTF format.

### Value

A GRanges.

### Examples

```
dir            <- system.file("extdata", package="srnadiff", mustWork = TRUE)
gffFile       <- file.path(dir, "mirbase21_GRCh38.gff3")
annotation <- readMiRBasePreAnnotation(gffFile)
```

---

readWholeGenomeAnnotation

*Segmentation using an annotation file that contains every genomic feature; it extracts the miRNAs.*

---

### Description

Segmentation using an annotation file that contains every genomic feature; it extracts the miRNAs.

### Usage

```
readWholeGenomeAnnotation(fileName)
```

### Arguments

fileName            The annotation file name in GFF/GTF format.

### Value

A GRanges.



**Examples**

```
dir      <- system.file("extdata", package="srnadiff", mustWork = TRUE)
gtfFile  <- file.path(dir, "Homo_sapiens.GRCh38.76.gtf.gz")
annotation <- readWholeGenomeAnnotation(gtfFile)
```

---

regions

*Get the output regions*

---

**Description**

Get the output regions

**Usage**

```
regions(object, pvalue = 0.05)

## S4 method for signature 'sRNADiff,numeric'
regions(object, pvalue = 0.05)

## S4 method for signature 'sRNADiff,ANY'
regions(object)
```

**Arguments**

object            An srnadiff object.  
pvalue            A minimum p-value

**Value**

The selected regions

**Examples**

```
exp <- sRNADiffExample()
regions(exp)
```

---

runAll

*Run the segmentation using 3 different methods, and reconcile them.*

---

**Description**

Run the segmentation using 3 different methods, and reconcile them.

**Usage**

```
runAll(object)
```

**Arguments**

object            An srnadiff object.

**Value**

A GRanges.

**Examples**

```
exp            <- sRNADiffExample()
exp            <- runAll(exp)
```

---

runAllAnnotation            *Segmentation using an annotation file.*

---

**Description**

Segmentation using an annotation file.

**Usage**

```
runAllAnnotation(object)
```

**Arguments**

object            An srnadiff object.

**Value**

A GRanges.

---

runAllHmm            *Segmentation of the genome using an HMM.*

---

**Description**

Segmentation of the genome using an HMM.

**Usage**

```
runAllHmm(object)
```

**Arguments**

object            An srnadiff object.

**Value**

A GRanges object.

---

runAllNaive	<i>Segmentation of the genome in a naive way.</i>
-------------	---

---

**Description**

Segmentation of the genome in a naive way.

**Usage**

```
runAllNaive(object)
```

**Arguments**

object            An srnadiff object.

**Value**

A GRanges.

---

runAllSlice	<i>Segmentation of the genome using a slice method.</i>
-------------	---

---

**Description**

Segmentation of the genome using a slice method.

**Usage**

```
runAllSlice(object)
```

**Arguments**

object            An srnadiff object.

**Value**

A GRanges object.

---

runHmm	<i>Initialize and run the HMM.</i>
--------	------------------------------------

---

**Description**

Initialize and run the HMM.

**Usage**

```
runHmm(object, counts, pvalues)
```

**Arguments**

object	An srnadiff object.
counts	The counts: a list of vectors or integers.
pvalues	The p-values: a list of numeric.

**Value**

A GRanges.

---

runSlice	<i>Initialize and run the slice method.</i>
----------	---

---

**Description**

Initialize and run the slice method.

**Usage**

```
runSlice(object)
```

**Arguments**

object	An srnadiff object.
--------	---------------------

**Value**

A GRanges.

---

```
setEmissionProbabilities
```

*Set emission probabilities (for the HMM step): probability to have a p-value not less than a threshold in the "not-differentially expressed" state, and a p-value not greater than this threshold in the "differentially expressed" state (supposed equal).*

---

### Description

Set emission probabilities (for the HMM step): probability to have a p-value not less than a threshold in the "not-differentially expressed" state, and a p-value not greater than this threshold in the "differentially expressed" state (supposed equal).

### Usage

```
setEmissionProbabilities(object, probability)

## S4 method for signature 'sRNADiff,numeric'
setEmissionProbabilities(object, probability)
```

### Arguments

object	An srnadiff object.
probability	The emission probability

### Value

The same object

### Examples

```
exp <- sRNADiffExample()
exp <- setEmissionProbabilities(exp, 0.9)
```

---

```
setEmissionThreshold
```

*Set emission threshold (for the HMM step): the emission distribution being binomial, all the p-values less than this threshold belong to one class, and all the p-values greater than this threshold belong to the other class.*

---

### Description

Set emission threshold (for the HMM step): the emission distribution being binomial, all the p-values less than this threshold belong to one class, and all the p-values greater than this threshold belong to the other class.

**Usage**

```
setEmissionThreshold(object, threshold)

## S4 method for signature 'sRNADiff,numeric'
setEmissionThreshold(object, threshold)
```

**Arguments**

object            An srnadiff object.  
threshold        The emission threshold

**Value**

The same object

**Examples**

```
exp <- sRNADiffExample()
exp <- setEmissionThreshold(exp, 0.1)
```

---

setMergeDistance        *Set the threshold to merge close regions (in the naive step)*

---

**Description**

Set the threshold to merge close regions (in the naive step)

**Usage**

```
setMergeDistance(object, distance)

## S4 method for signature 'sRNADiff,numeric'
setMergeDistance(object, distance)
```

**Arguments**

object            An srnadiff object.  
distance        The maximum distance

**Value**

The same object

**Examples**

```
exp <- sRNADiffExample()
exp <- setMergeDistance(exp, 1000)
```

---

setMinDepth	<i>Set min minimum depth to localize regions</i>
-------------	--

---

**Description**

Set min minimum depth to localize regions

**Usage**

```
setMinDepth(object, depth)
```

```
## S4 method for signature 'sRNADiff,numeric'  
setMinDepth(object, depth)
```

**Arguments**

object	An srnadiff object.
depth	The minimum depth

**Value**

The same object

**Examples**

```
exp <- sRNADiffExample()  
exp <- setMinDepth(exp, 3)
```

---

setMinDifferences	<i>Set the threshold to remove similar regions (in the slice step)</i>
-------------------	--

---

**Description**

Set the threshold to remove similar regions (in the slice step)

**Usage**

```
setMinDifferences(object, differences)
```

```
## S4 method for signature 'sRNADiff,numeric'  
setMinDifferences(object, differences)
```

**Arguments**

object	An srnadiff object.
differences	The minimum number of different nt.

**Value**

The same object

**Examples**

```
exp <- sRNADiffExample()
exp <- setMinDifferences(exp, 10)
```

---

setMinOverlap	<i>Set minimum overlap (for the last quantification step): all the reads with at least n nucleotides shared with a feature will be used for quantification of this feature.</i>
---------------	---

---

**Description**

Set minimum overlap (for the last quantification step): all the reads with at least n nucleotides shared with a feature will be used for quantification of this feature.

**Usage**

```
setMinOverlap(object, minOverlap)

## S4 method for signature 'sRNADiff,numeric'
setMinOverlap(object, minOverlap)
```

**Arguments**

object	An srnadiff object.
minOverlap	The minimum overlap

**Value**

The same object

**Examples**

```
exp <- sRNADiffExample()
exp <- setMinOverlap(exp, 10)
```



---

setNThreads	<i>Set number of threads to use</i>
-------------	-------------------------------------

---

**Description**

Set number of threads to use

**Usage**

```
setNThreads(object, nThreads)
```

```
## S4 method for signature 'srNADiff,numeric'
setNThreads(object, nThreads)
```

**Arguments**

object	An srnadiff object.
nThreads	The number of threads

**Value**

The same object

**Examples**

```
exp <- srNADiffExample()
exp <- setNThreads(exp, 4)
```

---

setSizes	<i>Set min and max sizes of the regions</i>
----------	---

---

**Description**

Set min and max sizes of the regions

**Usage**

```
setSizes(object, minSize, maxSize)
```

```
## S4 method for signature 'srNADiff,numeric,numeric'
setSizes(object, minSize, maxSize)
```

**Arguments**

object	An srnadiff object.
minSize	The minimum size.
maxSize	The maximum size.

**Value**

The same object

**Examples**

```
exp <- sRNADiffExample()
exp <- setSizes(exp, 10, 1000)
regions(exp)
```

---

setStrategies	<i>Set the different steps</i>
---------------	--------------------------------

---

**Description**

Set the different steps

**Usage**

```
setStrategies(object, annotation, naive, hmm, slice)

## S4 method for signature 'sRNADiff,logical,logical,logical,logical'
setStrategies(object,
  annotation, naive, hmm, slice)
```

**Arguments**

object	An srnadiff object.
annotation	The annotation step.
naive	The naive step.
hmm	The HMM step.
slice	The slice step.

**Value**

The same object

**Examples**

```
exp <- sRNADiffExample()
exp <- setStrategies(exp, TRUE, FALSE, TRUE, TRUE)
```

---

```
setTransitionProbabilities
    Set transition probabilities (for the HMM step).
```

---

**Description**

Set transition probabilities (for the HMM step).

**Usage**

```
setTransitionProbabilities(object, noDiffToDiff, diffToNoDiff)

## S4 method for signature 'sRNADiff,numeric,numeric'
setTransitionProbabilities(object,
    noDiffToDiff, diffToNoDiff)
```

**Arguments**

object	An srnadiff object.
noDiffToDiff	probability to change from the "not-differentially expressed" state to the "differentially expressed" state
diffToNoDiff	probability to change from the "differentially expressed" state to the "not-differentially expressed" state

**Value**

The same object

**Examples**

```
exp <- sRNADiffExample()
exp <- setTransitionProbabilities(exp, 0.001, 0.000001)
```

---

```
show,sRNADiff-method  Overloading the show method
```

---

**Description**

Overloading the show method

**Usage**

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

**Arguments**

object	An srnadiff object.
--------	---------------------

**Value**

A description of the object.

**Examples**

```
exp <- sRNADiffExample()
exp
```

---

srnadiff	<i>srnadiff: A package for differential expression of sRNA-Seq.</i>
----------	---

---

**Description**

The srnadiff package provides uses four strategies to find differentially expressed loci.

**Author(s)**

Matthias Zytnecki, <matthias.zytnecki@inra.fr>

---

sRNADiff-class	<i>An S4 class to represent sRNA-Seq data for differential expression.</i>
----------------	--

---

**Description**

An S4 class to represent sRNA-Seq data for differential expression.

**Slots**

**annotation** The annotation in GRanges format.  
**bamFileNames** The name of one read file in BAM format.  
**bamFiles** The BAM files in a BamFileList.  
**chromosomes** The names of the chromosomes.  
**chromosomeSizes** The sizes of the chromosomes.  
**replicates** The names of the replicates.  
**conditions** The condition to which each replicate belongs.  
**coverages** The coverages, a vector of RLE.  
**lengths** The lengths parts of the coverages.  
**values** The values parts of the coverages.  
**design** Experimental design, a DataFrame for DESeq2  
**regions** A GenomicRanges of the possibly differentially expressed region  
**minDepth** Minimum depth to consider to find regions  
**minSize** Minimum region size  
**maxSize** Maximum region size  
**mergeDistance** Distance to merge consecutive region

minDifferences Minimum number of different nt between two regions  
 noDiffToDiff Transition probability  
 diffToNoDiff Transition probability  
 emission Emission probability  
 emissionThreshold Emission threshold  
 skipAnnotation Whether to skip the annotation strategy step  
 skipNaive Whether to skip the naive strategy step  
 skipHmm Whether to skip the HMM strategy step  
 skipSlice Whether to skip the slice strategy step  
 nThreads Number of threads

---

sRNADiffExample	<i>Example constructor</i>
-----------------	----------------------------

---

### Description

Example constructor

### Usage

```
sRNADiffExample()
```

### Value

An srnadiff object

### Examples

```
exp <- sRNADiffExample()
```

---

sRNADiffExp	<i>Constructor.</i>
-------------	---------------------

---

### Description

Constructor.

### Usage

```
sRNADiffExp(annotation = NULL, bamFileNames, replicates, conditions,
  lazyload = FALSE)
```

**Arguments**

annotation	The GRanges annotation
bamFileNames	The name of one read file in BAM format.
replicates	The names of the replicates.
conditions	The condition to which each replicate belongs.
lazyload	Usual for S4 functions.

**Value**

An sRNADiff object.

**Examples**

```
dir      <- system.file("extdata", package="srnadiff", mustWork = TRUE)
data     <- read.csv(file.path(dir, "data.csv"))
gtfFile  <- file.path(dir, "Homo_sapiens.GRCh38.76.gtf.gz")
annotation <- readWholeGenomeAnnotation(gtfFile)
bamFiles <- file.path(dir, data$FileName)
replicates <- data$SampleName
conditions <- factor(data$Condition)
exp      <- sRNADiffExp(annotation, bamFiles, replicates, conditions)
```

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