# Package 'Signac'

August 21, 2024

```
Title Analysis of Single-Cell Chromatin Data
```

**Version** 1.14.0 **Date** 2024-08-21

**Description** A framework for the analysis and exploration of single-cell chromatin data. The 'Signac' package contains functions for quantifying single-cell chromatin data, computing per-cell quality control metrics, dimension reduction and normalization, visualization, and DNA sequence motif analysis. Reference: Stuart et al. (2021) <doi:10.1038/s41592-021-01282-5>.

**Depends** R (>= 4.1.0), methods **License** MIT + file LICENSE

Encoding UTF-8 LazyData true RoxygenNote 7.3.2

URL https://github.com/stuart-lab/signac, https://stuartlab.org/signac

BugReports https://github.com/stuart-lab/signac/issues

LinkingTo Rcpp

Imports GenomeInfoDb (>= 1.29.3), GenomicRanges, IRanges, Matrix, Rsamtools, S4Vectors, SeuratObject (>= 5.0.2), data.table, dplyr (>= 1.0.0), future, future.apply, ggplot2, rlang, irlba, pbapply, tidyr, patchwork, stats, utils, BiocGenerics, stringi, fastmatch, RcppRoll, scales, Rcpp, grid, tidyselect, vctrs, lifecycle

Collate 'RcppExports.R' 'data.R' 'differential\_accessibility.R' 'generics.R' 'dimension\_reduction.R' 'footprinting.R' 'fragments.R' 'genomeinfodb-methods.R' 'granges-methods.R' 'heatmaps.R' 'iranges-methods.R' 'links.R' 'mito.R' 'motifs.R' 'objects.R' 'peaks.R' 'preprocessing.R' 'quantification.R' 'region-enrichment.R' 'utilities.R' 'visualization.R' 'zzz.R'

Suggests Seurat (>= 5.0.2), ggforce, ggrepel, ggseqlogo, testthat (>= 2.1.0), chromVAR, SummarizedExperiment, TFBSTools, motifmatchr, BSgenome, shiny, miniUI, rtracklayer, biovizBase, Biostrings, lsa, MASS

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Author Tim Stuart [aut, cre] (<a href="https://orcid.org/0000-0002-3044-0897">https://orcid.org/0000-0002-3044-0897</a>),
Avi Srivastava [aut] (<a href="https://orcid.org/0000-0001-9798-2079">https://orcid.org/0000-0001-9798-2079</a>),
Paul Hoffman [ctb] (<a href="https://orcid.org/0000-0002-7693-8957">https://orcid.org/0000-0001-9448-8833</a>))
Rahul Satija [ctb] (<a href="https://orcid.org/0000-0001-9448-8833">https://orcid.org/0000-0001-9448-8833</a>)

Maintainer Tim Stuart <stuartt@gis.a-star.edu.sg>

Repository CRAN

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Signac-package Signac: Analysis of Single-Cell Chromatin Data

# Description

A framework for the analysis and exploration of single-cell chromatin data. The 'Signac' package contains functions for quantifying single-cell chromatin data, computing per-cell quality control metrics, dimension reduction and normalization, visualization, and DNA sequence motif analysis. Reference: Stuart et al. (2021) doi:10.1038/s41592021012825.

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#### Author(s)

**Maintainer**: Tim Stuart <stuartt@gis.a-star.edu.sg> (ORCID)

Authors:

• Avi Srivastava <asrivastava@nygenome.org> (ORCID)

Other contributors:

- Paul Hoffman <phoffman@nygenome.org> (ORCID) [contributor]
- Rahul Satija <rsatija@nygenome.org> (ORCID) [contributor]

#### See Also

Useful links:

- https://github.com/stuart-lab/signac
- https://stuartlab.org/signac
- Report bugs at https://github.com/stuart-lab/signac/issues

AccessiblePeaks

Accessible peaks

### **Description**

Find accessible peaks in a set of cells

#### Usage

```
AccessiblePeaks(
  object,
  assay = NULL,
  idents = NULL,
  cells = NULL,
  min.cells = 10
)
```

#### **Arguments**

object	A Seurat object
assay	Name of assay to use
idents	A set of identity classes to find accessible peaks for
cells	A vector of cells to find accessible peaks for
min.cells	Minimum number of cells with the peak accessible (>0 counts) for the peak to
	be called accessible

#### Value

Returns a vector of peak names

6 AddMotifs

AddChromatinModule Add chromatin module

# Description

Compute chromVAR deviations for groups of peaks. The goal of this function is similar to that of AddModuleScore except that it is designed for single-cell chromatin data. The chromVAR deviations for each group of peaks will be added to the object metadata.

### Usage

```
AddChromatinModule(object, features, genome, assay = NULL, verbose = TRUE, ...)
```

#### **Arguments**

object A Seurat object

features A named list of features to include in each module. The name of each element

in the list will be used to name the modules computed, which will be stored in

the object metadata.

genome A BSgenome object

assay Name of assay to use. If NULL, use the default assay.

verbose Display messages

... Additional arguments passed to RunChromVAR

#### Value

Returns a Seurat object

AddMotifs Add DNA sequence motif information

#### **Description**

Construct a Motif object containing DNA sequence motif information and add it to an existing Seurat object or ChromatinAssay. If running on a Seurat object, AddMotifs will also run RegionStats to compute the GC content of each peak and store the results in the feature metadata. PFMs or PWMs are matched to the genome sequence using the matchMotifs function with default parameters to construct a matrix of motif positions in genomic regions.

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#### Usage

```
AddMotifs(object, ...)
## Default S3 method:
AddMotifs(object, genome, pfm, verbose = TRUE, ...)
## S3 method for class 'ChromatinAssay'
AddMotifs(object, genome, pfm, verbose = TRUE, ...)
## S3 method for class 'Assay'
AddMotifs(object, genome, pfm, verbose = TRUE, ...)
## S3 method for class 'StdAssay'
AddMotifs(object, genome, pfm, verbose = TRUE, ...)
## S3 method for class 'Seurat'
AddMotifs(object, genome, pfm, assay = NULL, verbose = TRUE, ...)
```

### **Arguments**

object	A Seurat object or ChromatinAssay object
	Additional arguments passed to other methods
genome	A BSgenome, DNAStringSet, FaFile, or string stating the genome build recognized by getBSgenome.
pfm	A PFMatrixList or PWMatrixList object containing position weight/frequency matrices to use
verbose	Display messages
assay	Name of assay to use. If NULL, use the default assay

#### Value

When running on a ChromatinAssay or Seurat object, returns a modified version of the input object. When running on a matrix, returns a Motif object.

#### See Also

### motifmatchr

### **Description**

Quantifies fragment counts per cell in fixed-size genome bins across the whole genome, then removes bins with less than a desired minimum number of counts in the bin, then merges adjacent tiles into a single region.

8 AggregateTiles

### Usage

```
AggregateTiles(object, ...)
## S3 method for class 'Seurat'
AggregateTiles(
 object,
  genome,
  assay = NULL,
 new.assay.name = "tiles",
 min_counts = 5,
 binsize = 5000,
 verbose = TRUE,
)
## S3 method for class 'ChromatinAssay'
AggregateTiles(
 object,
  genome,
 min_counts = 5,
 binsize = 5000,
 verbose = TRUE,
)
## Default S3 method:
AggregateTiles(
 object,
 genome,
 cells = NULL,
 min_counts = 5,
 binsize = 5000,
 verbose = TRUE,
  . . .
)
```

# Arguments

binsize

object	A Seurat object or ChromatinAssay object
	Additional arguments passed to other methods
genome	genome A vector of chromosome sizes for the genome. This is used to construct the genome bin coordinates. The can be obtained by calling seqlengths on a BSgenome-class object.
assay	Name of assay to use
new.assay.name	Name of new assay to create containing aggregated genome tiles
min_counts	Minimum number of counts for a tile to be retained prior to aggregation

Size of the genome bins (tiles) in base pairs

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verbose	Display messages
cells	Cells to include

#### Value

When running on a Seurat object, returns the Seurat object with a new ChromatinAssay added.

When running on a ChromatinAssay, returns a new ChromatinAssay containing the aggregated genome tiles.

When running on a fragment file, returns a sparse region x cell matrix.

AlleleFreq

Compute allele frequencies per cell

### Description

Collapses allele counts for each strand and normalize by the total number of counts at each nucleotide position.

#### Usage

```
AlleleFreq(object, ...)
## Default S3 method:
AlleleFreq(object, variants, ...)
## S3 method for class 'Assay'
AlleleFreq(object, variants, ...)
## S3 method for class 'StdAssay'
AlleleFreq(object, variants, ...)
## S3 method for class 'Seurat'
AlleleFreq(object, variants, assay = NULL, new.assay.name = "alleles", ...)
```

# Arguments

object A Seurat object, Assay, or matrix
... Arguments passed to other methods

variants A character vector of informative variants to keep. For example, c("627G>A", "709G>A", "1045G>A", "17

assay Name of assay to use

new.assay.name Name of new assay to store variant data in

#### Value

Returns a Seurat object with a new assay containing the allele frequencies for the informative variants.

10 Annotation

Annotation

Annotation

#### Description

Get the annotation from a ChromatinAssay

#### Usage

```
Annotation(object, ...)

Annotation(object, ...) <- value

## S3 method for class 'ChromatinAssay'
Annotation(object, ...)

## S3 method for class 'Seurat'
Annotation(object, ...)

## S3 replacement method for class 'ChromatinAssay'
Annotation(object, ...) <- value

## S3 replacement method for class 'Seurat'
Annotation(object, ...) <- value
```

#### Arguments

object A Seurat object or ChromatinAssay object

... Arguments passed to other methods

value A value to set. Can be NULL, to remove the current annotation information, or a

GRanges object. If a GRanges object is supplied and the genome information is stored in the assay, the genome of the new annotations must match the genome

of the assay.

#### Value

Returns a GRanges object if the annotation data is present, otherwise returns NULL

### **Examples**

```
Annotation(atac_small[["peaks"]])
Annotation(atac_small)
genes <- Annotation(atac_small)
Annotation(atac_small[["peaks"]]) <- genes</pre>
```

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```
genes <- Annotation(atac_small)
Annotation(atac_small) <- genes</pre>
```

AnnotationPlot

Plot gene annotations

### Description

Display gene annotations in a given region of the genome.

### Usage

```
AnnotationPlot(
  object,
  region,
  assay = NULL,
  mode = "gene",
  sep = c("-", "-"),
  extend.upstream = 0,
  extend.downstream = 0)
```

### Arguments

object A Seurat object A genomic region to plot region Name of assay to use. If NULL, use the default assay. assay mode Display mode. Choose either "gene" or "transcript" to determine whether genes or transcripts are plotted. Separators to use for strings encoding genomic coordinates. First element is sep used to separate the chromosome from the coordinates, second element is used to separate the start from end coordinate. extend.upstream Number of bases to extend the region upstream. extend.downstream

Number of bases to extend the region downstream.

#### Value

```
Returns a ggplot object
```

#### **Examples**

```
AnnotationPlot(object = atac_small, region = c("chr1-29554-39554"))
```

12 as.ChromatinAssay

as.ChromatinAssay

Convert objects to a ChromatinAssay

### **Description**

Convert objects to a ChromatinAssay

# Usage

```
as.ChromatinAssay(x, ...)
## S3 method for class 'Assay'
as.ChromatinAssay(
    x,
    ranges = NULL,
    seqinfo = NULL,
    annotation = NULL,
    motifs = NULL,
    fragments = NULL,
    bias = NULL,
    positionEnrichment = NULL,
    sep = c("-", "-"),
    ...
)
```

#### **Arguments**

x An object to convert to class ChromatinAssay

... Arguments passed to other methods

ranges A GRanges object

seqinfo A Seqinfo object containing basic information about the genome used. Al-

ternatively, the name of a UCSC genome can be provided and the sequence

information will be downloaded from UCSC.

annotation Genomic annotation

motifs A Motif object

fragments A list of Fragment objects bias Tn5 integration bias matrix

positionEnrichment

A named list of position enrichment matrices.

sep Characters used to separate the chromosome, start, and end coordinates in the

row names of the data matrix

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atac\_small

A small example scATAC-seq dataset

#### **Description**

A subsetted version of 10x Genomics 10k human (hg19) PBMC scATAC-seq dataset

### Usage

atac\_small

### **Format**

A Seurat object with the following assays

peaks A peak x cell dataset

**bins** A 5 kb genome bin x cell dataset

**RNA** A gene x cell dataset

#### **Source**

```
https://support.10xgenomics.com/single-cell-atac/datasets/1.1.0/atac_v1_pbmc_10k
```

AverageCounts

Average Counts

### **Description**

Compute the mean counts per group of cells for a given assay

### Usage

```
AverageCounts(object, assay = NULL, group.by = NULL, verbose = TRUE)
```

### Arguments

object A Seurat object

assay Name of assay to use. Default is the active assay

group.by Grouping variable to use. Default is the active identities

verbose Display messages

#### Value

Returns a dataframe

### **Examples**

AverageCounts(atac\_small)

14 BigwigTrack

BigwigTrack

Plot data from BigWig files

#### Description

Create coverage tracks, heatmaps, or line plots from bigwig files.

### Usage

```
BigwigTrack(
  region,
  bigwig,
  smooth = 200,
  extend.upstream = 0,
  extend.downstream = 0,
  type = "coverage",
  y_label = "bigWig",
  bigwig.scale = "common",
  ymax = NULL,
  max.downsample = 3000,
  downsample.rate = 0.1
)
```

### **Arguments**

region GRanges object specifying region to plot

bigwig List of bigwig file paths. List should be named, and the name of each element

in the list of files will be displayed alongside the track in the final plot.

smooth Number of bases to smooth data over (rolling mean). If NULL, do not apply

smoothing.

extend.upstream

Number of bases to extend the region upstream.

extend.downstream

Number of bases to extend the region downstream.

type Plot type. Can be one of "line", "heatmap", or "coverage"

y\_label Y-axis label

bigwig. scale Scaling to apply to data from different bigwig files. Can be:

- common: plot each bigwig on a common scale (default)
- separate: plot each bigwig on a separate scale ranging from zero to the maximum value for that bigwig file within the plotted region

ymax Maximum value for Y axis. Can be one of:

• NULL: set to the highest value among all the tracks (default)

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> • qXX: clip the maximum value to the XX quantile (for example, q95 will set the maximum value to 95% of the maximum value in the data). This can help remove the effect of extreme values that may otherwise distort the scale.

• numeric: manually define a Y-axis limit

max.downsample Minimum number of positions kept when downsampling. Downsampling rate is adaptive to the window size, but this parameter will set the minimum possible number of positions to include so that plots do not become too sparse when the window size is small.

downsample.rate

Fraction of positions to retain when downsampling. Retaining more positions can give a higher-resolution plot but can make the number of points large, resulting in larger file sizes when saving the plot and a longer period of time needed to draw the plot.

#### **Details**

Note that this function does not work on windows.

#### Value

Returns a ggplot object

BinarizeCounts

Binarize counts

### **Description**

Set counts >1 to 1 in a count matrix

# Usage

```
BinarizeCounts(object, ...)
## Default S3 method:
BinarizeCounts(object, assay = NULL, verbose = TRUE, ...)
## S3 method for class 'Assay'
BinarizeCounts(object, assay = NULL, verbose = TRUE, ...)
## S3 method for class 'Seurat'
BinarizeCounts(object, assay = NULL, verbose = TRUE, ...)
```

blacklist\_ce10

#### **Arguments**

object A Seurat object

... Arguments passed to other methods

assay Name of assay to use. Can be a list of assays, and binarization will be applied

to each.

verbose Display messages

### Value

Returns a Seurat object

# **Examples**

```
x <- matrix(data = sample(0:3, size = 25, replace = TRUE), ncol = 5)
BinarizeCounts(x)
BinarizeCounts(atac_small[['peaks']])
BinarizeCounts(atac_small)</pre>
```

blacklist\_ce10

Genomic blacklist regions for C. elegans ce10 (0-based)

# Description

Genomic blacklist regions for C. elegans ce10 (0-based)

### Usage

```
blacklist_ce10
```

### **Format**

A GRanges object

```
https://github.com/Boyle-Lab/Blacklist
doi:10.1038/s4159801945839z
```

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blacklist\_ce11

Genomic blacklist regions for C. elegans cell (0-based)

# Description

Genomic blacklist regions for C. elegans ce11 (0-based)

### Usage

blacklist\_ce11

#### **Format**

A GRanges object

#### **Source**

```
https://github.com/Boyle-Lab/Blacklist
doi:10.1038/s4159801945839z
```

blacklist\_dm3

Genomic blacklist regions for Drosophila dm3 (0-based)

# Description

Genomic blacklist regions for Drosophila dm3 (0-based)

# Usage

blacklist\_dm3

#### **Format**

A GRanges object

```
https://github.com/Boyle-Lab/Blacklist
doi:10.1038/s4159801945839z
```

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blacklist\_dm6

Genomic blacklist regions for Drosophila dm6 (0-based)

# Description

Genomic blacklist regions for Drosophila dm6 (0-based)

### Usage

blacklist\_dm6

#### **Format**

A GRanges object

#### **Source**

```
https://github.com/Boyle-Lab/Blacklist
doi:10.1038/s4159801945839z
```

blacklist\_hg19

Genomic blacklist regions for Human hg19 (0-based)

# Description

Genomic blacklist regions for Human hg19 (0-based)

# Usage

blacklist\_hg19

#### **Format**

A GRanges object

```
https://github.com/Boyle-Lab/Blacklist
doi:10.1038/s4159801945839z
```

blacklist\_hg38

blacklist\_hg38

Genomic blacklist regions for Human GRCh38

# Description

Genomic blacklist regions for Human GRCh38

# Usage

blacklist\_hg38

#### **Format**

A GRanges object

#### **Source**

```
https://github.com/Boyle-Lab/Blacklistdoi:10.1038/s4159801945839z
```

blacklist\_hg38\_unified

Unified genomic blacklist regions for Human GRCh38

# Description

Manually curated genomic blacklist regions for the hg38 genome by Anshul Kundaje and Anna Shcherbina. See https://www.encodeproject.org/files/ENCFF356LFX/ for a description of how this blacklist was curated.

### Usage

```
blacklist_hg38_unified
```

#### **Format**

A GRanges object

# Author(s)

Anshul Kundaje Anna Shcherbina

```
https://www.encodeproject.org/files/ENCFF356LFX/doi:10.1038/s4159801945839z
```

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blacklist\_mm10

Genomic blacklist regions for Mouse mm10 (0-based)

### **Description**

Genomic blacklist regions for Mouse mm10 (0-based)

#### Usage

```
blacklist_mm10
```

### **Format**

A GRanges object

#### **Source**

```
https://github.com/Boyle-Lab/Blacklist
doi:10.1038/s4159801945839z
```

CallPeaks

Call peaks

### **Description**

Call peaks using MACS. Fragment files linked to the specified assay will be used to call peaks. If multiple fragment files are present, all will be used in a single MACS invocation. Returns the .narrowPeak MACS output as a GRanges object.

### Usage

```
CallPeaks(object, ...)
## S3 method for class 'Seurat'
CallPeaks(
  object,
  assay = NULL,
  group.by = NULL,
  idents = NULL,
  macs2.path = NULL,
  broad = FALSE,
  format = "BED",
  outdir = tempdir(),
  fragment.tempdir = tempdir(),
  combine.peaks = TRUE,
```

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```
effective.genome.size = 2.7e+09,
  extsize = 200,
  shift = -extsize/2,
  additional.args = NULL,
  name = Project(object),
  cleanup = TRUE,
  verbose = TRUE,
)
## S3 method for class 'ChromatinAssay'
CallPeaks(
  object,
 macs2.path = NULL,
  outdir = tempdir(),
  broad = FALSE,
  format = "BED",
  effective.genome.size = 2.7e+09,
  extsize = 200,
  shift = -extsize/2,
  additional.args = NULL,
  name = "macs2",
  cleanup = TRUE,
  verbose = TRUE,
)
## S3 method for class 'Fragment'
CallPeaks(
 object,
 macs2.path = NULL,
  outdir = tempdir(),
  broad = FALSE,
  format = "BED",
  effective.genome.size = 2.7e+09,
  extsize = 200,
  shift = -extsize/2,
  additional.args = NULL,
  name = "macs2",
  cleanup = TRUE,
  verbose = TRUE,
)
## Default S3 method:
CallPeaks(
  object,
 macs2.path = NULL,
```

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```
outdir = tempdir(),
broad = FALSE,
format = "BED",
effective.genome.size = 2.7e+09,
extsize = 200,
shift = -extsize/2,
additional.args = NULL,
name = "macs2",
cleanup = TRUE,
verbose = TRUE,
...
)
```

#### **Arguments**

object A Seurat object, ChromatinAssay object, Fragment object, or the path to frag-

ment file/s.

... Arguments passed to other methods

assay Name of assay to use

group.by Grouping variable to use. If set, peaks will be called independently on each

group of cells and then combined. Note that to call peaks using subsets of cells we first split the fragment file/s used, so using a grouping variable will require extra time to split the files and perform multiple MACS peak calls, and will store additional files on-disk that may be large. Note that we store split fragment files in the temp directory (tempdir) by default, and if the program is interrupted before completing these temporary files will not be removed. If NULL, peaks

are called using all cells together (pseudobulk).

idents List of identities to include if grouping cells (only valid if also setting the group.by

parameter). If NULL, peaks will be called for all cell identities.

macs2.path Path to MACS program. If NULL, try to find MACS automatically.

broad Call broad peaks (--broad parameter for MACS)

format File format to use. Should be either "BED" or "BEDPE" (see MACS documen-

tation).

outdir Path for output files

fragment.tempdir

Path to write temporary fragment files. Only used if group. by is not NULL.

combine.peaks

Controls whether peak calls from different groups of cells are combined using GenomicRanges::reduce when calling peaks for different groups of cells (group.by parameter). If FALSE, a list of GRanges object will be returned. Note that metadata fields such as the p-value, q-value, and fold-change information

for each peak will be lost if combining peaks.

effective.genome.size

Effective genome size parameter for MACS (-g). Default is the human effective

genome size (2.7e9).

extsize extsize parameter for MACS. Only relevant if format="BED"

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shift shift parameter for MACS. Only relevant if format="BED"

additional.args

Additional arguments passed to MACS. This should be a single character string

name Name for output MACS files. This will also be placed in the name field in the

GRanges output.

cleanup Remove MACS output files

verbose Display messages

#### **Details**

```
See https://macs3-project.github.io/MACS/ for MACS documentation. If you call peaks using MACS2 please cite: doi:10.1186/gb200899r137
```

#### Value

Returns a GRanges object

Cells.Fragment

Set and get cell barcode information for a Fragment object

#### **Description**

This returns the names of cells in the object that are contained in the fragment file. These cell barcodes may not match the barcodes present in the fragment file. The Fragment object contains an internal mapping of the cell names in the ChromatinAssay object to the cell names in the fragment file, so that cell names can be changed in the assay without needing to change the cell names on disk.

#### Usage

```
## S3 method for class 'Fragment'
Cells(x, ...)
## S3 replacement method for class 'Fragment'
Cells(x, ...) <- value</pre>
```

# **Arguments**

x A Fragment object

... Arguments passed to other methods

value A vector of cell names to store in the Fragment object

#### **Details**

To access the cell names that are stored in the fragment file itself, use GetFragmentData(object = x, name = "cells").

24 CellsPerGroup

Cells<-

Set and get cell barcode information for a Fragment object

### **Description**

Set and get cell barcode information for a Fragment object

# Usage

```
Cells(x, ...) \leftarrow value
```

### **Arguments**

x A Seurat object

... Arguments passed to other methods value A character vector of cell barcodes

CellsPerGroup

Cells per group

# Description

Count the number of cells in each group

# Usage

```
CellsPerGroup(object, group.by = NULL)
```

# Arguments

object A Seurat object

group.by A grouping variable. Default is the active identities

### Value

Returns a vector

### **Examples**

```
CellsPerGroup(atac_small)
```

ChromatinAssay-class 25

ChromatinAssay-class The ChromatinAssay class

### **Description**

The ChromatinAssay object is an extended Assay for the storage and analysis of single-cell chromatin data.

#### **Slots**

ranges A GRanges object describing the genomic location of features in the object

motifs A Motif object

fragments A list of Fragment objects.

seqinfo A Seqinfo object containing basic information about the genome sequence used.

annotation A GRanges object containing genomic annotations. This should be a GRanges object with the following columns:

- tx\_id: Transcript ID
- gene\_name: Gene name
- gene\_id: Gene ID
- gene\_biotype: Gene biotype (e.g. "protein\_coding", "lincRNA")
- type: Annotation type (e.g. "exon", "gap")

bias A vector containing Tn5 integration bias information (frequency of Tn5 integration at different kmers)

positionEnrichment A named list of matrices containing positional enrichment scores for Tn5 integration (for example, enrichment at the TSS)

links A GRanges object describing linked genomic positions, such as co-accessible sites or enhancergene regulatory relationships. This should be a GRanges object, where the start and end coordinates are the two linked genomic positions, and must contain a "score" metadata column.

 ${\tt ClosestFeature}$ 

Closest Feature

#### **Description**

Find the closest feature to a given set of genomic regions

### Usage

```
ClosestFeature(object, regions, annotation = NULL, ...)
```

26 ClusterClonotypes

#### **Arguments**

object A Seurat object

regions A set of genomic regions to query

annotation A GRanges object containing annotation information. If NULL, use the annota-

tions stored in the object.

... Additional arguments passed to StringToGRanges

#### Value

Returns a dataframe with the name of each region, the closest feature in the annotation, and the distance to the feature.

### **Examples**

```
ClosestFeature(
  object = atac_small,
  regions = head(granges(atac_small))
)
```

ClusterClonotypes

Find relationships between clonotypes

### **Description**

Perform hierarchical clustering on clonotype data

# Usage

```
ClusterClonotypes(object, assay = NULL, group.by = NULL)
```

# Arguments

object A Seurat object
assay Name of assay to use
group.by Grouping variable for cells

#### Value

Returns a list containing two objects of class hclust, one for the cell clustering and one for the feature (allele) clustering

CombineTracks 27

CombineTracks Combine genome region plots
---

### **Description**

This can be used to combine coverage plots, peak region plots, gene annotation plots, and linked element plots. The different tracks are stacked on top of each other and the x-axis combined.

#### Usage

```
CombineTracks(plotlist, expression.plot = NULL, heights = NULL, widths = NULL)
```

#### **Arguments**

plotlist A list of plots to combine. Must be from the same genomic region.

expression.plot

Plot containing gene expression information. If supplied, this will be placed to the left of the coverage tracks and aligned with each track

heights Relative heights for each plot. If NULL, the first plot will be 8x the height of the other tracks.

widths Relative widths for each plot. Only required if adding a gene expression panel. If NULL, main plots will be 8x the width of the gene expression panel

#### Value

Returns a patchworked ggplot2 object

#### **Examples**

```
p1 <- PeakPlot(atac_small, region = "chr1-29554-39554")
p2 <- AnnotationPlot(atac_small, region = "chr1-29554-39554")
CombineTracks(plotlist = list(p1, p2), heights = c(1, 1))</pre>
```

ConnectionsToLinks

Cicero connections to links

### **Description**

Convert the output of Cicero connections to a set of genomic ranges where the start and end coordinates of the range are the midpoints of the linked elements. Only elements on the same chromosome are included in the output.

#### Usage

```
ConnectionsToLinks(conns, ccans = NULL, threshold = 0, sep = c("-", "-"))
```

28 ConvertMotifID

#### **Arguments**

conns A dataframe containing co-accessible elements. This would usually be the out-

put of run\_cicero or assemble\_connections. Specifically, this should be a dataframe where the first column contains the genomic coordinates of the first element in the linked pair of elements, with chromosome, start, end coordinates separated by "-" characters. The second column should be the second element in the linked pair, formatted in the same way as the first column. A third column

should contain the co-accessibility scores.

ccans This is optional, but if supplied should be a dataframe containing the cis-co-

accessibility network (CCAN) information generated by generate\_ccans. Specifically, this should be a dataframe containing the name of the peak in the first

column, and the CCAN that it belongs to in the second column.

threshold Threshold for retaining a coaccessible site. Links with a value less than or equal

to this threshold will be discarded.

sep Separators to use for strings encoding genomic coordinates. First element is

used to separate the chromosome from the coordinates, second element is used

to separate the start from end coordinate.

#### Details

See the Cicero package for more information: https://bioconductor.org/packages/cicero/

#### Value

Returns a GRanges object

ConvertMotifID

Convert between motif name and motif ID

### **Description**

Converts from motif name to motif ID or vice versa. To convert common names to IDs, use the name parameter. To convert IDs to common names, use the id parameter.

#### Usage

```
ConvertMotifID(object, ...)
## Default S3 method:
ConvertMotifID(object, name, id, ...)
## S3 method for class 'Motif'
ConvertMotifID(object, ...)
## S3 method for class 'ChromatinAssay'
ConvertMotifID(object, ...)
```

CountFragments 29

```
## S3 method for class 'Assay'
ConvertMotifID(object, ...)
## S3 method for class 'StdAssay'
ConvertMotifID(object, ...)
## S3 method for class 'Seurat'
ConvertMotifID(object, assay = NULL, ...)
```

# Arguments

object A Seurat, ChromatinAssay, or Motif object
... Arguments passed to other methods
name A vector of motif names
id A vector of motif IDs. Only one of name and id should be supplied

assay For Seurat object. Name of assay to use. If NULL, use the default assay

#### Value

Returns a character vector with the same length and order as the input. Any names or IDs that were not found will be stored as NA.

CountFragments	Count fragments

### **Description**

Count total fragments per cell barcode present in a fragment file.

#### Usage

```
CountFragments(fragments, cells = NULL, max_lines = NULL, verbose = TRUE)
```

### **Arguments**

fragments	Path to a fragment file. If a list of fragment files is provided, the total fragments for each cell barcode across all files will be returned
cells	Cells to include. If NULL, include all cells
max_lines	Maximum number of lines to read from the fragment file. If NULL, read all lines in the file.
verbose	Display messages

30 CountsInRegion

#### Value

Returns a data.frame with the following columns:

- CB: the cell barcode
- frequency\_count: total number of fragments sequenced for the cell
- mononucleosome: total number of fragments with length between 147 bp and 294 bp
- nucleosome\_free: total number of fragments with length <147 bp
- reads\_count: total number of reads sequenced for the cell

# **Examples**

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
counts <- CountFragments(fragments = fpath)</pre>
```

CountsInRegion

Counts in region

### **Description**

Count reads per cell overlapping a given set of regions

### Usage

```
CountsInRegion(object, assay, regions, ...)
```

#### **Arguments**

```
object A Seurat object
assay Name of a chromatin assay in the object to use
regions A GRanges object
... Additional arguments passed to findOverlaps
```

#### Value

Returns a numeric vector

### **Examples**

```
CountsInRegion(
  object = atac_small,
  assay = 'bins',
  regions = blacklist_hg19
)
```

```
coverage, ChromatinAssay-method
```

Coverage of a ChromatinAssay object

# Description

This is the coverage method for ChromatinAssay objects.

# Usage

```
## S4 method for signature 'ChromatinAssay'
coverage(
    x,
    shift = 0L,
    width = NULL,
    weight = 1L,
    method = c("auto", "sort", "hash")
)

## S4 method for signature 'Seurat'
coverage(
    x,
    shift = 0L,
    width = NULL,
    weight = 1L,
    method = c("auto", "sort", "hash")
)
```

### **Arguments**

Х	A ChromatinAssay object
shift	How much each range should be shifted before coverage is computed. See coverage in the <b>IRanges</b> package.
width	Specifies the length of the returned coverage vectors. See coverage in the <b>IRanges</b> package.
weight	Assigns weight to each range in x. See coverage in the <b>IRanges</b> package.
method	See coverage in the <b>IRanges</b> package

### **Functions**

- coverage(ChromatinAssay): method for ChromatinAssay objects
- coverage(Seurat): method for Seurat objects

#### See Also

- coverage-methods in the **IRanges** package.
- coverage-methods in the GenomicRanges package
- ChromatinAssay-class

CoverageBrowser Genome browser

### **Description**

Interactive version of the CoveragePlot function. Allows altering the genome position interactively. The current view at any time can be saved to a list of ggplot objects using the "Save plot" button, and this list of plots will be returned after ending the browser by pressing the "Done" button.

#### **Usage**

```
CoverageBrowser(object, region, assay = NULL, sep = c("-", "-"), ...)
```

# Arguments

object	A Seurat object
region	A set of genomic coordinates
assay	Name of assay to use
sep	Separators for genomic coordinates if region supplied as a string rather than GRanges object
	Parameters passed to CoveragePlot

#### Value

Returns a list of ggplot objects

CoveragePlot Plot Tn5 insertion frequency over a region

# Description

Plot frequency of Tn5 insertion events for different groups of cells within given regions of the genome. Tracks are normalized using a per-group scaling factor computed as the number of cells in the group multiplied by the mean sequencing depth for that group of cells. This accounts for differences in number of cells and potential differences in sequencing depth between groups.

#### Usage

```
CoveragePlot(
  object,
  region,
  features = NULL,
  assay = NULL,
  split.assays = FALSE,
  assay.scale = "common",
  show.bulk = FALSE,
  expression.assay = "RNA",
  expression.slot = "data",
  annotation = TRUE,
  peaks = TRUE,
  peaks.group.by = NULL,
  ranges = NULL,
  ranges.group.by = NULL,
  ranges.title = "Ranges",
  region.highlight = NULL,
  links = TRUE,
  tile = FALSE,
  tile.size = 100,
  tile.cells = 100,
  bigwig = NULL,
  bigwig.type = "coverage",
  bigwig.scale = "common",
  heights = NULL,
  group.by = NULL,
  split.by = NULL,
 window = 100,
  extend.upstream = 0,
  extend.downstream = 0,
  scale.factor = NULL,
 ymax = NULL,
  cells = NULL,
  idents = NULL,
  sep = c("-", "-"),
 max.downsample = 3000,
  downsample.rate = 0.1,
)
```

### **Arguments**

object A Seurat object

region

A set of genomic coordinates to show. Can be a GRanges object, a string encoding a genomic position, a gene name, or a vector of strings describing the genomic coordinates or gene names to plot. If a gene name is supplied, annotations must be present in the assay.

features A vector of features present in another assay to plot alongside accessibility

tracks (for example, gene names).

Name of the assay to plot. If a list of assays is provided, data from each assay assay

will be shown overlaid on each track. The first assay in the list will define the assay used for gene annotations, links, and peaks (if shown). The order of assays

given defines the plotting order.

split.assays When plotting data from multiple assays, display each assay as a separate track.

If FALSE, data from different assays are overlaid on a single track with transparancy

applied.

assay.scale Scaling to apply to data from different assays. Can be:

• common: plot all assays on a common scale (default)

• separate: plot each assay on a separate scale ranging from zero to the maximum value for that assay within the plotted region

show.bulk Include coverage track for all cells combined (pseudo-bulk). Note that this will plot the combined accessibility for all cells included in the plot (rather than all

cells in the object).

expression.assay

Name of the assay containing expression data to plot alongside accessibility tracks. Only needed if supplying features argument.

expression.slot

Name of slot to pull expression data from. Only needed if supplying the features

argument.

Display gene annotations. Set to TRUE or FALSE to control whether genes

models are displayed, or choose "transcript" to display all transcript isoforms,

or "gene" to display gene models only (same as setting TRUE).

Display peaks

peaks.group.by Grouping variable to color peaks by. Must be a variable present in the feature

metadata. If NULL, do not color peaks by any variable.

ranges Additional genomic ranges to plot

ranges.group.by

Grouping variable to color ranges by. Must be a variable present in the metadata stored in the ranges genomic ranges. If NULL, do not color by any variable.

Y-axis title for ranges track. Only relevant if ranges parameter is set. ranges.title

region.highlight

Region to highlight on the plot. Should be a GRanges object containing the coordinates to highlight. By default, regions will be highlighted in grey. To change the color of the highlighting, include a metadata column in the GRanges

object named "color" containing the color to use for each region.

Display links. This can be a TRUE/FALSE value which will determine whether a links track is displayed, and if TRUE links for all genes in the plotted region will be shown. Alternatively, a character vector can be provided, giving a list of gene names to plot links for. If this is provided, only links for those genes will

be displayed in the plot.

annotation

peaks

links

Display per-cell fragment information in sliding windows. If plotting multi-

tile

assay data, only the first assay is shown in the tile plot. tile.size Size of the sliding window for per-cell fragment tile plot tile.cells Number of cells to display fragment information for in tile plot. List of bigWig file paths to plot data from. Files can be remotely hosted. The bigwig name of each element in the list will determine the y-axis label given to the track. bigwig.type Type of track to use for bigWig files ("line", "heatmap", or "coverage"). Should either be a single value, or a list of values giving the type for each individual track in the provided list of bigwig files. bigwig.scale Same as assay.scale parameter, except for bigWig files when plotted with bigwig.type="coverage" Relative heights for each track (accessibility, gene annotations, peaks, links). heights group.by Name of one or more metadata columns to group (color) the cells by. Default is the current cell identities A metadata variable to split the tracks by. For example, grouping by "celltype" split.by and splitting by "batch" will create separate tracks for each combination of celltype and batch. window Smoothing window size extend.upstream Number of bases to extend the region upstream. extend.downstream Number of bases to extend the region downstream. scale.factor Scaling factor for track height. If NULL (default), use the median group scaling factor determined by total number of fragments sequences in each group. Maximum value for Y axis. Can be one of: ymax • NULL: set to the highest value among all the tracks (default) • qXX: clip the maximum value to the XX quantile (for example, q95 will set the maximum value to 95% of the maximum value in the data). This can help remove the effect of extreme values that may otherwise distort the scale. • numeric: manually define a Y-axis limit cells Which cells to plot. Default all cells

idents Which identities to include in the plot. Default is all identities.

sep Separators to use for strings encoding genomic coordinates. First element is

used to separate the chromosome from the coordinates, second element is used

to separate the start from end coordinate.

max.downsample Minimum number of positions kept when downsampling. Downsampling rate

is adaptive to the window size, but this parameter will set the minimum possible number of positions to include so that plots do not become too sparse when the

window size is small.

downsample.rate

Fraction of positions to retain when downsampling. Retaining more positions can give a higher-resolution plot but can make the number of points large, resulting in larger file sizes when saving the plot and a longer period of time needed to draw the plot.

.. Additional arguments passed to wrap\_plots

#### **Details**

Additional information can be layered on the coverage plot by setting several different options in the CoveragePlot function. This includes showing:

- · gene annotations
- · peak positions
- additional genomic ranges
- additional data stored in a bigWig file, which may be hosted remotely
- gene or protein expression data alongside coverage tracks
- peak-gene links
- the position of individual sequenced fragments as a heatmap
- data for multiple chromatin assays simultaneously
- · a pseudobulk for all cells combined

#### Value

Returns a patchwork object

# Examples

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")</pre>
fragments <- CreateFragmentObject(</pre>
 path = fpath,
 cells = colnames(atac_small),
 validate.fragments = FALSE
Fragments(atac_small) <- fragments</pre>
# Basic coverage plot
CoveragePlot(object = atac_small, region = c("chr1-713500-714500"))
# Show additional ranges
ranges.show <- StringToGRanges("chr1-713750-714000")</pre>
CoveragePlot(object = atac_small, region = c("chr1-713500-714500"), ranges = ranges.show)
# Highlight region
CoveragePlot(object = atac_small, region = c("chr1-713500-714500"), region.highlight = ranges.show)
# Change highlight color
ranges.show$color <- "orange"</pre>
CoveragePlot(object = atac_small, region = c("chr1-713500-714500"), region.highlight = ranges.show)
```

CreateChromatinAssay

```
# Show expression data
CoveragePlot(object = atac_small, region = c("chr1-713500-714500"), features = "ELK1")
```

CreateChromatinAssay Object

### **Description**

Create a ChromatinAssay object from a count matrix or normalized data matrix. The expected format of the input matrix is features x cells. A set of genomic ranges must be supplied along with the matrix, with the length of the ranges equal to the number of rows in the matrix. If a set of genomic ranges are not supplied, they will be extracted from the row names of the matrix.

### Usage

```
CreateChromatinAssay(
  counts,
  data,
 min.cells = 0,
 min.features = 0,
 max.cells = NULL,
  ranges = NULL,
  motifs = NULL,
  fragments = NULL,
  genome = NULL,
  annotation = NULL,
  bias = NULL,
  positionEnrichment = NULL,
  sep = c("-", "-"),
  validate.fragments = TRUE,
  verbose = TRUE,
)
```

#### **Arguments**

counts	Unnormalized data (raw counts)
data	Normalized data; if provided, do not pass counts
min.cells	Include features detected in at least this many cells. Will subset the counts matrix as well. To reintroduce excluded features, create a new object with a lower cutoff.
min.features	Include cells where at least this many features are detected.

max.cells Include features detected in less than this many cells. Will subset the counts

matrix as well. To reintroduce excluded features, create a new object with a higher cutoff. This can be useful for chromatin assays where certain artefactual loci accumulate reads in all cells. A percentage cutoff can also be set using 'q' followed by the percentage of cells, for example 'q90' will discard features detected in 90 percent of cells. If NULL (default), do not apply any maximum

value.

ranges A set of GRanges corresponding to the rows of the input matrix

motifs A Motif object (not required)

fragments Path to a tabix-indexed fragments file for the data contained in the input matrix.

If multiple fragment files are required, you can add additional Fragment object to the assay after it is created using the CreateFragmentObject and Fragments

functions. Alternatively, a list of Fragment objects can be provided.

genome A Seqinfo object containing basic information about the genome used. Al-

ternatively, the name of a UCSC genome can be provided and the sequence

information will be downloaded from UCSC.

annotation A set of GRanges containing annotations for the genome used

bias A Tn5 integration bias matrix

positionEnrichment

A named list of matrices containing positional signal enrichment information for

each cell. Should be a cell x position matrix, centered on an element of interest

(for example, TSS sites).

sep Separators to use for strings encoding genomic coordinates. First element is

used to separate the chromosome from the coordinates, second element is used

to separate the start from end coordinate. Only used if ranges is NULL.

validate.fragments

Check that cells in the assay are present in the fragment file.

verbose Display messages

... Additional arguments passed to CreateFragmentObject

CreateFragmentObject Create a Fragment object

#### Description

Create a Fragment object to store fragment file information. This object stores a 32-bit MD5 hash of the fragment file and the fragment file index so that any changes to the files on-disk can be detected. A check is also performed to ensure that the expected cells are present in the fragment file.

CreateMotifMatrix 39

#### Usage

```
CreateFragmentObject(
  path,
  cells = NULL,
  validate.fragments = TRUE,
  verbose = TRUE,
  ...
)
```

#### **Arguments**

path

A path to the fragment file. The file should contain a tabix index in the same directory.

cells

A named character vector containing cell barcodes contained in the fragment file. This does not need to be all cells in the fragment file, but there should be no cells in the vector that are not present in the fragment file. A search of the file will be performed until at least one fragment from each cell is found. If NULL, don't check for expected cells.

Each element of the vector should be a cell barcode that appears in the fragment file, and the name of each element should be the corresponding cell name in the object.

validate.fragments

Check that expected cells are present in the fragment file.

verbose

Display messages

. . .

Additional arguments passed to ValidateCells

#### **Examples**

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
cells <- colnames(x = atac_small)
names(x = cells) <- paste0("test_", cells)
frags <- CreateFragmentObject(path = fpath, cells = cells, verbose = FALSE, tolerance = 0.5)</pre>
```

CreateMotifMatrix

Create motif matrix

### **Description**

Create a motif x feature matrix from a set of genomic ranges, the genome, and a set of position weight matrices.

40 CreateMotifMatrix

### Usage

```
CreateMotifMatrix(
  features,
  pwm,
  genome,
  score = FALSE,
  use.counts = FALSE,
  sep = c("-", "-"),
  ...
)
```

# Arguments

features	A GRanges object containing a set of genomic features
pwm	$A \ PFMatrixList \ or \ PWMatrixList \ object \ containing \ position \ weight/frequency \\ matrices \ to \ use$
genome	Any object compatible with the genome argument in matchMotifs
score	Record the motif match score, rather than presence/absence (default FALSE)
use.counts	Record motif counts per region. If FALSE (default), record presence/absence of motif. Only applicable if score=FALSE.
sep	A length-2 character vector containing the separators to be used when constructing matrix rownames from the GRanges
	Additional arguments passed to matchMotifs

#### **Details**

Requires that motifmatchr is installed https://www.bioconductor.org/packages/motifmatchr/.

#### Value

Returns a sparse matrix

```
## Not run:
library(JASPAR2018)
library(TFBSTools)
library(BSgenome.Hsapiens.UCSC.hg19)

pwm <- getMatrixSet(
   x = JASPAR2018,
   opts = list(species = 9606, all_versions = FALSE)
)
motif.matrix <- CreateMotifMatrix(
  features = granges(atac_small),
   pwm = pwm,
   genome = BSgenome.Hsapiens.UCSC.hg19
)</pre>
```

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```
## End(Not run)
```

CreateMotifObject

Create motif object

### Description

Create a Motif-class object.

### Usage

```
CreateMotifObject(
  data = NULL,
  pwm = NULL,
  motif.names = NULL,
  positions = NULL,
  meta.data = NULL
)
```

# Arguments

data A motif x region matrix

pwm A named list of position weight matrices or position frequency matrices match-

ing the motif names in data. Can be of class PFMatrixList.

motif.names A named list of motif names. List element names must match the names given

in pwm. If NULL, use the names from the list of position weight or position frequency matrices. This can be used to set a alternative common name for the motif. If a PFMatrixList is passed to pwm, it will pull the motif name from the

PFMatrixList.

positions A GRangesList object containing exact positions of each motif.

meta.data A data.frame containing metadata

#### Value

Returns a Motif object

```
motif.matrix <- matrix(
  data = sample(c(0,1),
    size = 100,
    replace = TRUE),
  ncol = 5
)
motif <- CreateMotifObject(data = motif.matrix)</pre>
```

DepthCor

y		
---	--	--

# Description

Create a scatterplot using variables in the object metadata and color cells by the density of points in the x-y space.

### Usage

```
DensityScatter(object, x, y, log_x = FALSE, log_y = FALSE, quantiles = NULL)
```

## Arguments

object	A Seurat object
X	Name of metadata variable to plot on x axis
у	Name of metadata variable to plot on y axis
log_x	log10 transform x values
log_y	log10 transform y values
quantiles	Vector of quantiles to display for x and y data distribution. Must be integer values between 0 and 100. TRUE can be passed as a shorthand way to set c(5, 10, 90, 95). If FALSE or NULL, no quantile information is displayed

### Value

Returns a ggplot object

### **Description**

Compute the correlation between total counts and each reduced dimension component.

### Usage

```
DepthCor(object, assay = NULL, reduction = "lsi", n = 10, ...)
```

# Arguments

object	A Seurat object
assay	Name of assay to use for sequencing depth. If NULL, use the default assay.
reduction	Name of a dimension reduction stored in the input object
n	Number of components to use. If NULL, use all components.
	Additional arguments passed to cor

DownsampleFeatures 43

### Value

```
Returns a ggplot object
```

# **Examples**

```
DepthCor(object = atac_small)
```

DownsampleFeatures

Downsample Features

# Description

Randomly downsample features and assign to VariableFeatures for the object. This will select n features at random.

# Usage

```
DownsampleFeatures(object, assay = NULL, n = 20000, verbose = TRUE)
```

# Arguments

object A Seurat object

assay Name of assay to use. Default is the active assay.

n Number of features to retain (default 20000).

verbose Display messages

#### Value

Returns a Seurat object with VariableFeatures set to the randomly sampled features.

```
DownsampleFeatures(atac_small, n = 10)
```

44 Extend

ExpressionPlot Plot gene expression

# Description

Display gene expression values for different groups of cells and different genes. Genes will be arranged on the x-axis and different groups stacked on the y-axis, with expression value distribution for each group shown as a violin plot. This is designed to work alongside a genomic coverage track, and the plot will be able to be aligned with coverage tracks for the same groups of cells.

# Usage

```
ExpressionPlot(
  object,
  features,
  assay = NULL,
  group.by = NULL,
  idents = NULL,
  slot = "data"
)
```

# Arguments

object A Seurat object

features A list of features to plot

assay Name of the assay storing expression information

group.by A grouping variable to group cells by. If NULL, use the current cell identities idents A list of identities to include in the plot. If NULL, include all identities

#### **Examples**

slot

```
ExpressionPlot(atac_small, features = "TSPAN6", assay = "RNA")
```

Which slot to pull expression data from

Extend Extend

### **Description**

Resize GenomicRanges upstream and or downstream. From https://support.bioconductor.org/p/78652/

FeatureMatrix 45

#### Usage

```
Extend(x, upstream = 0, downstream = 0, from.midpoint = FALSE)
```

#### **Arguments**

x A range

upstream Length to extend upstream
downstream Length to extend downstream

from.midpoint Count bases from region midpoint, rather than the 5' or 3' end for upstream and

downstream respectively.

#### Value

Returns a GRanges object

### **Examples**

```
Extend(x = blacklist_hg19, upstream = 100, downstream = 100)
```

FeatureMatrix

Feature Matrix

### **Description**

Construct a feature x cell matrix from a genomic fragments file

#### Usage

```
FeatureMatrix(
  fragments,
  features,
  cells = NULL,
  process_n = 2000,
  sep = c("-", "-"),
  verbose = TRUE
)
```

### **Arguments**

fragments

A list of Fragment objects. Note that if setting the cells parameter, the requested cells should be present in the supplied Fragment objects. However, if the cells information in the fragment object is not set (Cells(fragments) is NULL), then the fragment object will still be searched.

46 FilterCells

features	A GRanges object containing a set of genomic intervals. These will form the rows of the matrix, with each entry recording the number of unique reads falling in the genomic region for each cell. If a genomic region provided is on a chromosome that is not present in the fragment file, it will not be included in the returned matrix.
cells	Vector of cells to include. If NULL, include all cells found in the fragments file
process_n	Number of regions to load into memory at a time, per thread. Processing more regions at once can be faster but uses more memory.
sep	Vector of separators to use for genomic string. First element is used to separate chromosome and coordinates, second separator is used to separate start and end coordinates.
verbose	Display messages

# Value

Returns a sparse matrix

### **Examples**

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
fragments <- CreateFragmentObject(fpath)
FeatureMatrix(
   fragments = fragments,
   features = granges(atac_small)
)</pre>
```

FilterCells

Filter cells from fragment file

# Description

Remove all fragments that are not from an allowed set of cell barcodes from the fragment file. This will create a new file on disk that only contains fragments from cells specified in the cells argument. The output file is block gzip-compressed and indexed, ready for use with Signac functions.

### Usage

```
FilterCells(
  fragments,
  cells,
  outfile = NULL,
  buffer_length = 256L,
  verbose = TRUE
)
```

FindClonotypes 47

### **Arguments**

fragments Path to a fragment file cells A vector of cells to keep outfile Name for output file

buffer\_length Size of buffer to be read from the fragment file. This must be longer than the

longest line in the file.

verbose Display messages

### **Examples**

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
tmpf <- tempfile(fileext = ".gz")
FilterCells(
   fragments = fpath,
   cells = head(colnames(atac_small)),
   outfile = tmpf
)
file.remove(tmpf)</pre>
```

FindClonotypes

Find clonotypes

#### **Description**

Identify groups of related cells from allele frequency data. This will cluster the cells based on their allele frequencies, reorder the factor levels for the cluster identities by hierarchical clustering the collapsed (pseudobulk) cluster allele frequencies, and set the variable features for the allele frequency assay to the order of features defined by hierarchical clustering.

#### Usage

```
FindClonotypes(
  object,
  assay = NULL,
  features = NULL,
  metric = "cosine",
  resolution = 1,
  k = 10,
  algorithm = 3
)
```

### **Arguments**

object A Seurat object
assay Name of assay to use

features Features to include when constructing neighbor graph

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metric Distance metric to use

resolution Clustering resolution to use. See FindClusters k Passed to k.param argument in FindNeighbors

algorithm Community detection algorithm to use. See FindClusters

#### Value

Returns a Seurat object

FindMotifs

**FindMotifs** 

#### **Description**

Find motifs over-represented in a given set of genomic features. Computes the number of features containing the motif (observed) and compares this to the total number of features containing the motif (background) using the hypergeometric test.

#### Usage

```
FindMotifs(
  object,
  features,
  background = 40000,
  assay = NULL,
  verbose = TRUE,
  p.adjust.method = "BH",
  ...
)
```

## **Arguments**

object A Seurat object

features A vector of features to test for enrichments over background

background Either a vector of features to use as the background set, or a number specify

the number of features to randomly select as a background set. If a number is provided, regions will be selected to match the sequence characteristics of the query features. To match the sequence characteristics, these characteristics must be stored in the feature metadata for the assay. This can be added using the

RegionStats function. If NULL, use all features in the assay.

assay Which assay to use. Default is the active assay

verbose Display messages

p.adjust.method

Multiple testing correction method to be applied. Passed to p. adjust.

.. Arguments passed to MatchRegionStats.

findOverlaps-methods 49

#### Value

Returns a data frame

#### **Examples**

```
de.motif <- head(rownames(atac_small))
bg.peaks <- tail(rownames(atac_small))
FindMotifs(
  object = atac_small,
  features = de.motif,
  background = bg.peaks
)</pre>
```

findOverlaps-methods Find overlapping ranges for ChromatinAssay objects

### Description

The findOverlaps, countOverlaps methods are available for ChromatinAssay objects. This allows finding overlaps between genomic ranges and the ranges stored in the ChromatinAssay.

### Usage

```
## S4 method for signature 'Vector, ChromatinAssay'
findOverlaps(
  query,
  subject,
 maxgap = -1L,
 minoverlap = 0L,
  type = c("any", "start", "end", "within", "equal"),
  select = c("all", "first", "last", "arbitrary"),
  ignore.strand = FALSE
)
## S4 method for signature 'ChromatinAssay, Vector'
findOverlaps(
  query,
  subject,
 maxgap = -1L,
 minoverlap = 0L,
  type = c("any", "start", "end", "within", "equal"),
  select = c("all", "first", "last", "arbitrary"),
  ignore.strand = FALSE
)
## S4 method for signature 'ChromatinAssay, ChromatinAssay'
findOverlaps(
```

```
query,
  subject,
  maxgap = -1L,
 minoverlap = 0L,
  type = c("any", "start", "end", "within", "equal"),
  select = c("all", "first", "last", "arbitrary"),
  ignore.strand = FALSE
)
## S4 method for signature 'Vector, Seurat'
findOverlaps(
  query,
  subject,
 maxgap = -1L,
 minoverlap = 0L,
  type = c("any", "start", "end", "within", "equal"),
  select = c("all", "first", "last", "arbitrary"),
  ignore.strand = FALSE
)
## S4 method for signature 'Seurat, Vector'
findOverlaps(
  query,
  subject,
 maxgap = -1L,
 minoverlap = 0L,
  type = c("any", "start", "end", "within", "equal"),
  select = c("all", "first", "last", "arbitrary"),
  ignore.strand = FALSE
)
## S4 method for signature 'Seurat, Seurat'
findOverlaps(
  query,
  subject,
 maxgap = -1L,
 minoverlap = 0L,
  type = c("any", "start", "end", "within", "equal"),
  select = c("all", "first", "last", "arbitrary"),
  ignore.strand = FALSE
## S4 method for signature 'Vector, ChromatinAssay'
countOverlaps(
  query,
  subject,
  maxgap = -1L,
 minoverlap = 0L,
```

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```
type = c("any", "start", "end", "within", "equal"),
  ignore.strand = FALSE
)
## S4 method for signature 'ChromatinAssay, Vector'
countOverlaps(
 query,
  subject,
 maxgap = -1L,
 minoverlap = 0L,
  type = c("any", "start", "end", "within", "equal"),
  ignore.strand = FALSE
)
## S4 method for signature 'ChromatinAssay, ChromatinAssay'
countOverlaps(
  query,
  subject,
 maxgap = -1L,
 minoverlap = 0L,
type = c("any", "start", "end", "within", "equal"),
  ignore.strand = FALSE
)
## S4 method for signature 'Seurat, Vector'
countOverlaps(
  query,
  subject,
 maxgap = -1L,
 minoverlap = 0L,
  type = c("any", "start", "end", "within", "equal"),
  ignore.strand = FALSE
)
## S4 method for signature 'Vector, Seurat'
countOverlaps(
  query,
  subject,
 maxgap = -1L,
 minoverlap = 0L,
  type = c("any", "start", "end", "within", "equal"),
  ignore.strand = FALSE
## S4 method for signature 'Seurat, Seurat'
countOverlaps(
  query,
  subject,
```

```
maxgap = -1L,
minoverlap = 0L,
type = c("any", "start", "end", "within", "equal"),
ignore.strand = FALSE
)
```

#### **Arguments**

```
query, subject A ChromatinAssay object
maxgap, minoverlap, type, select, ignore.strand
See ?findOverlaps in the GenomicRanges and IRanges packages.
```

#### **Details**

If a ChromatinAssay is set as the default assay in a Seurat object, you can also call findOverlaps directly on the Seurat object.

#### Value

See findOverlaps

#### **Functions**

- findOverlaps(query = ChromatinAssay, subject = Vector): method for ChromatinAssay, Vector
- findOverlaps(query = ChromatinAssay, subject = ChromatinAssay): method for ChromatinAssay, ChromatinAssay
- findOverlaps(query = Vector, subject = Seurat): method for Vector, Seurat
- findOverlaps(query = Seurat, subject = Vector): method for Seurat, Vector
- findOverlaps(query = Seurat, subject = Seurat): method for Seurat, Seurat
- countOverlaps(query = Vector, subject = ChromatinAssay): method for Vector, ChromatinAssay
- countOverlaps(query = ChromatinAssay, subject = Vector): method for ChromatinAssay, Vector
- countOverlaps(query = ChromatinAssay, subject = ChromatinAssay): method for ChromatinAssay, ChromatinAssay
- countOverlaps(query = Seurat, subject = Vector): method for Seurat, Vector
- countOverlaps(query = Vector, subject = Seurat): method for Vector, Seurat
- countOverlaps(query = Seurat, subject = Seurat): method for Seurat, Seurat

#### See Also

- findOverlaps-methods in the **IRanges** package.
- findOverlaps-methods in the GenomicRanges package
- ChromatinAssay-class

FindTopFeatures 53

FindTopFeatures

Find most frequently observed features

### **Description**

Find top features for a given assay based on total number of counts for the feature. Can specify a minimum cell count, or a lower percentile bound to determine the set of variable features. Running this function will store the total counts and percentile rank for each feature in the feature metadata for the assay. To only compute the feature metadata, without changing the variable features for the assay, set min.cutoff=NA.

#### Usage

```
FindTopFeatures(object, ...)
## Default S3 method:
FindTopFeatures(object, assay = NULL, min.cutoff = "q5", verbose = TRUE, ...)
## S3 method for class 'Assay'
FindTopFeatures(object, assay = NULL, min.cutoff = "q5", verbose = TRUE, ...)
## S3 method for class 'StdAssay'
FindTopFeatures(object, assay = NULL, min.cutoff = "q5", verbose = TRUE, ...)
## S3 method for class 'Seurat'
FindTopFeatures(object, assay = NULL, min.cutoff = "q5", verbose = TRUE, ...)
```

## **Arguments**

object A Seurat object

. . . Arguments passed to other methods

assay Name of assay to use

min.cutoff Cutoff for feature to be included in the VariableFeatures for the object. This

can be a percentile specified as 'q' followed by the minimum percentile, for example 'q5' to set the top 95% most common features as the VariableFeatures for the object. Alternatively, this can be an integer specifying the minimum number of counts for the feature to be included in the set of VariableFeatures. For example, setting to 10 will include features with >10 total counts in the set of VariableFeatures. If NULL, include all features in VariableFeatures. If NA, VariableFeatures will not be altered, and only the feature metadata will be

updated with the total counts and percentile rank for each feature.

verbose Display messages

#### Value

Returns a Seurat object

54 Footprint

#### **Examples**

```
FindTopFeatures(object = atac_small[['peaks']]['data'])
FindTopFeatures(object = atac_small[['peaks']])
FindTopFeatures(object = atac_small[['peaks']])
FindTopFeatures(atac_small)
```

Footprint

Transcription factor footprinting analysis

### **Description**

Compute the normalized observed/expected Tn5 insertion frequency for each position surrounding a set of motif instances.

#### Usage

```
Footprint(object, ...)
## S3 method for class 'ChromatinAssay'
Footprint(
  object,
  genome,
 motif.name = NULL,
  key = motif.name,
  regions = NULL,
  assay = NULL,
  upstream = 250,
  downstream = 250,
  compute.expected = TRUE,
  in.peaks = FALSE,
  verbose = TRUE,
)
## S3 method for class 'Seurat'
Footprint(
  object,
  genome,
  regions = NULL,
  motif.name = NULL,
  assay = NULL,
  upstream = 250,
  downstream = 250,
  in.peaks = FALSE,
  verbose = TRUE,
)
```

#### **Arguments**

object A Seurat or ChromatinAssay object
... Arguments passed to other methods

genome A BSgenome object or any other object supported by getSeq. Do showMethods("getSeq")

to get the list of all supported object types.

motif.name Name of a motif stored in the assay to footprint. If not supplied, must supply a

set of regions.

key Key to store positional enrichment information under.

regions A set of genomic ranges containing the motif instances. These should all be the

same width.

assay Name of assay to use

upstream Number of bases to extend upstream downstream Number of bases to extend downstream

compute.expected

Find the expected number of insertions at each position given the local DNA

sequence context and the insertion bias of Tn5

in.peaks Restrict motifs to those that fall in peaks

verbose Display messages

#### Value

Returns a Seurat object

FractionCountsInRegion

Fraction of counts in a genomic region

## Description

Find the fraction of counts per cell that overlap a given set of genomic ranges

## Usage

FractionCountsInRegion(object, regions, assay = NULL, ...)

#### **Arguments**

object A Seurat object

regions A GRanges object containing a set of genomic regions

assay Name of assay to use

... Additional arguments passed to CountsInRegion

56 FragmentHistogram

#### Value

Returns a numeric vector

# **Examples**

```
## Not run:
FractionCountsInRegion(
  object = atac_small,
  assay = 'bins',
  regions = blacklist_hg19
)
## End(Not run)
```

Fragment-class

The Fragment class

### **Description**

The Fragment class is designed to hold information needed for working with fragment files.

#### **Slots**

path Path to the fragment file on disk. See https://support.10xgenomics.com/single-cell-atac/software/pipelines/latest/output/fragments

hash A vector of two md5sums: first element is the md5sum of the fragment file, the second element is the md5sum of the index.

cells A named vector of cells where each element is the cell barcode as it appears in the fragment file, and the name of each element is the corresponding cell barcode as stored in the ChromatinAssay object.

FragmentHistogram

Plot fragment length histogram

### **Description**

Plot the frequency that fragments of different lengths are present for different groups of cells.

Fragments 57

### Usage

```
FragmentHistogram(
  object,
  assay = NULL,
  region = "chr1-1-2000000",
  group.by = NULL,
  cells = NULL,
  log.scale = FALSE,
  ...
)
```

### **Arguments**

object	A Seurat object
assay	Which assay to use. Default is the active assay.
region	Genomic range to use. Default is fist two megabases of chromosome 1. Can be a GRanges object, a string, or a vector of strings.
group.by	Name of one or more metadata columns to group (color) the cells by. Default is the current cell identities
cells	Which cells to plot. Default all cells
log.scale	Display Y-axis on log scale. Default is FALSE.
	Arguments passed to other functions

### Value

Returns a ggplot object

# **Examples**

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
Fragments(atac_small) <- CreateFragmentObject(
  path = fpath,
  cells = colnames(atac_small),
  validate.fragments = FALSE
)
FragmentHistogram(object = atac_small, region = "chr1-10245-780007")</pre>
```

Fragments

Get the Fragment objects

# Description

Get the Fragment objects

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#### Usage

```
Fragments(object, ...)
Fragments(object, ...) <- value

## S3 method for class 'ChromatinAssay'
Fragments(object, ...)

## S3 method for class 'Seurat'
Fragments(object, ...)

## S3 replacement method for class 'ChromatinAssay'
Fragments(object, ...) <- value

## S3 replacement method for class 'Seurat'
Fragments(object, ...) <- value</pre>
```

#### **Arguments**

object A Seurat object or ChromatinAssay object
... Arguments passed to other methods
value A Fragment object or list of Fragment objects

#### Value

Returns a list of Fragment objects. If there are no Fragment objects present, returns an empty list.

```
Fragments(atac_small[["peaks"]])
Fragments(atac_small)
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
fragments <- CreateFragmentObject(
   path = fpath,
   cells = colnames(atac_small),
   validate.fragments = FALSE
)
Fragments(atac_small[["bins"]]) <- fragments
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
fragments <- CreateFragmentObject(
   path = fpath,
   cells = colnames(atac_small),
   validate.fragments = FALSE
)
Fragments(atac_small) <- fragments</pre>
```

FRiP 59

FRiP

Calculate fraction of reads in peaks per cell

# Description

Calculate fraction of reads in peaks per cell

### Usage

```
FRiP(object, assay, total.fragments, col.name = "FRiP", verbose = TRUE)
```

### **Arguments**

object A Seurat object

assay Name of the assay containing a peak x cell matrix

total.fragments

Name of a metadata column containing the total number of sequenced fragments

for each cell. This can be computed using the CountFragments function.

col.name Name of column in metadata to store the FRiP information.

verbose Display messages

#### Value

Returns a Seurat object

### **Examples**

```
FRiP(object = atac_small, assay = 'peaks', total.fragments = "fragments")
```

GeneActivity

Create gene activity matrix

# Description

Compute counts per cell in gene body and promoter region.

60 GeneActivity

#### Usage

```
GeneActivity(
  object,
  assay = NULL,
  features = NULL,
  extend.upstream = 2000,
  extend.downstream = 0,
  biotypes = "protein_coding",
  max.width = 5e+05,
  process_n = 2000,
  gene.id = FALSE,
  verbose = TRUE
)
```

#### **Arguments**

object A Seurat object

assay Name of assay to use. If NULL, use the default assay

features Genes to include. If NULL, use all protein-coding genes in the annotations

stored in the object

extend.upstream

Number of bases to extend upstream of the TSS

extend.downstream

Number of bases to extend downstream of the TTS

biotypes Gene biotypes to include. If NULL, use all biotypes in the gene annotation.

max.width Maximum allowed gene width for a gene to be quantified. Setting this parameter

can avoid quantifying extremely long transcripts that can add a relatively long

amount of time. If NULL, do not filter genes based on width.

process\_n Number of regions to load into memory at a time, per thread. Processing more

regions at once can be faster but uses more memory.

gene.id Record gene IDs in output matrix rather than gene name.

verbose Display messages

#### Value

Returns a sparse matrix

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
fragments <- CreateFragmentObject(
  path = fpath,
  cells = colnames(atac_small),
  validate.fragments = FALSE
)
Fragments(atac_small) <- fragments
GeneActivity(atac_small)</pre>
```

GenomeBinMatrix 61

	GenomeBinMatrix	Genome bin matrix
--	-----------------	-------------------

# Description

Construct a bin x cell matrix from a fragments file.

# Usage

```
GenomeBinMatrix(
  fragments,
  genome,
  cells = NULL,
  binsize = 5000,
  process_n = 2000,
  sep = c("-", "-"),
  verbose = TRUE
)
```

# Arguments

fragments	Path to tabix-indexed fragments file or a list of Fragment objects
genome	A vector of chromosome sizes for the genome. This is used to construct the genome bin coordinates. The can be obtained by calling seqlengths on a BSgenome-class object.
cells	Vector of cells to include. If NULL, include all cells found in the fragments file
binsize	Size of the genome bins to use
process_n	Number of regions to load into memory at a time, per thread. Processing more regions at once can be faster but uses more memory.
sep	Vector of separators to use for genomic string. First element is used to separate chromosome and coordinates, second separator is used to separate start and end coordinates.
verbose	Display messages

# **Details**

This function bins the genome and calls Feature Matrix to construct a bin x cell matrix.

# Value

Returns a sparse matrix

GetCellsInRegion

## **Examples**

```
genome <- 780007
names(genome) <- 'chr1'
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
fragments <- CreateFragmentObject(fpath)
GenomeBinMatrix(
   fragments = fragments,
   genome = genome,
   binsize = 1000
)</pre>
```

GetCellsInRegion

Get cells in a region

# Description

Extract cell names containing reads mapped within a given genomic region

### Usage

```
GetCellsInRegion(tabix, region, cells = NULL)
```

### **Arguments**

tabix	Tabix object
region	A string giving the region to extract from the fragments file
cells	Vector of cells to include in output. If NULL, include all cells

#### Value

Returns a list

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
GetCellsInRegion(tabix = fpath, region = "chr1-10245-762629")</pre>
```

GetFootprintData 63

### **Description**

Extract footprint data for a set of transcription factors or metafeatures. This function will pull accessibility data for a given feature (eg, a TF), and perform background normalization for each identity class. This is the data that's used to create TF footprinting plots with the PlotFootprint function.

# Usage

```
GetFootprintData(
  object,
  features,
  assay = NULL,
  group.by = NULL,
  idents = NULL
)
```

#### Arguments

object A Seurat object
features A vector of features to extract data for
assay Name of assay to use
group.by A grouping variable
idents Set of identities to group cells by

#### Value

Returns a data.frame with the following columns:

- group: Cell group (determined by group.by parameter
- position: Position relative to motif center
- count: Normalized Tn5 insertion counts at each position
- norm.value: Normalized Tn5 insertion counts at each position (same as count)
- feature: Name of the footprinted motif
- · class: observed or expected

GetFragmentData

Get Fragment object data

# Description

Extract data from a Fragment-class object

### Usage

```
GetFragmentData(object, slot = "path")
```

### Arguments

object A Fragment object

slot Information to pull from object (path, hash, cells, prefix, suffix)

 ${\tt GetGRangesFromEnsDb}$ 

Extract genomic ranges from EnsDb object

# Description

Pulls the transcript information for all chromosomes from an EnsDb object. This wraps crunch and applies the extractor function to all chromosomes present in the EnsDb object.

### Usage

```
GetGRangesFromEnsDb(
  ensdb,
  standard.chromosomes = TRUE,
  biotypes = c("protein_coding", "lincRNA", "rRNA", "processed_transcript"),
  verbose = TRUE
)
```

#### **Arguments**

ensdb An EnsDb object

standard.chromosomes

Keep only standard chromosomes

biotypes Biotypes to keep verbose Display messages GetIntersectingFeatures

Find intersecting regions between two objects

#### **Description**

Intersects the regions stored in the rownames of two objects and returns a vector containing the names of rows that intersect for each object. The order of the row names return corresponds to the intersecting regions, i.e. the nth feature of the first vector will intersect the nth feature in the second vector. A distance parameter can be given, in which case features within the given distance will be called as intersecting.

# Usage

```
GetIntersectingFeatures(
  object.1,
  object.2,
  assay.1 = NULL,
  assay.2 = NULL,
  distance = 0,
  verbose = TRUE
)
```

### Arguments

```
object.1 The first Seurat object
object.2 The second Seurat object
assay.1 Name of the assay to use in the first object. If NULL, use the default assay
assay.2 Name of the assay to use in the second object. If NULL, use the default assay
distance Maximum distance between regions allowed for an intersection to be recorded.
Default is 0.
verbose Display messages
```

### Value

Returns a list of two character vectors containing the row names in each object that overlap each other.

```
GetIntersectingFeatures(
  object.1 = atac_small,
  object.2 = atac_small,
  assay.1 = 'peaks',
  assay.2 = 'bins'
)
```

66 GetLinkedPeaks

GetLinkedGenes Get genes linked to peaks

### **Description**

Retrieve peak-gene links for a given set of genes. Links must be first obtained by running the LinkPeaks function.

#### Usage

```
GetLinkedGenes(object, features, assay = NULL, min.abs.score = 0)
```

#### **Arguments**

object A Seurat object

features A list of peaks to find linked genes for

assay Name of assay to use. If NULL, use the default assay

min. abs. score Minimum absolute value of the link score for a link to be returned

#### **Details**

This function is designed to obtain the stored results from running the LinkPeaks function. Alternatively, custom peak-gene linkage methods can be used as long as they store the gene name, peak name, and a peak-gene score information as metadata columns named "gene," "peak," and "score" respectively.

#### See Also

GetLinkedPeaks

GetLinkedPeaks Get peaks linked to genes

### **Description**

Retrieve peak-gene links for a given set of genes. Links must be first obtained by running the LinkPeaks function.

### Usage

```
GetLinkedPeaks(object, features, assay = NULL, min.abs.score = 0)
```

GetMotifData 67

#### **Arguments**

A Seurat object object

features A list of genes to find linked peaks for

assay Name of assay to use. If NULL, use the default assay

min.abs.score Minimum absolute value of the link score for a link to be returned

#### **Details**

This function is designed to obtain the stored results from running the LinkPeaks function. Alternatively, custom peak-gene linkage methods can be used as long as they store the gene name, peak name, and a peak-gene score information as metadata columns named "gene," "peak," and "score" respectively.

### See Also

GetLinkedGenes

GetMotifData Retrieve a motif matrix

### **Description**

Get motif matrix for given assay

#### Usage

```
GetMotifData(object, ...)
## S3 method for class 'Motif'
GetMotifData(object, slot = "data", ...)
## S3 method for class 'ChromatinAssay'
GetMotifData(object, slot = "data", ...)
## S3 method for class 'Seurat'
GetMotifData(object, assay = NULL, slot = "data", ...)
```

### **Arguments**

object	A Seurat object
	Arguments passed to other methods
slot	Information to pull from object (data, pwm, meta.data)
assay	Which assay to use. Default is the current active assay

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#### Value

Returns a Seurat object

#### **Examples**

```
motif.obj <- SeuratObject::GetAssayData(
  object = atac_small[['peaks']], slot = "motifs"
)
GetMotifData(object = motif.obj)
GetMotifData(object = atac_small)</pre>
```

GetTSSPositions

Find transcriptional start sites

#### **Description**

Get the TSS positions from a set of genomic ranges containing gene positions. Ranges can contain exons, introns, UTRs, etc, rather than the whole transcript. Only protein coding gene biotypes are included in output.

#### Usage

```
GetTSSPositions(ranges, biotypes = "protein_coding")
```

# **Arguments**

ranges A GRanges object containing gene annotations.

biotypes Gene biotypes to include. If NULL, use all biotypes in the supplied gene anno-

tation.

granges-methods

Access genomic ranges for ChromatinAssay objects

#### **Description**

Methods for accessing GRanges object information stored in a ChromatinAssay object.

# Usage

```
## S4 method for signature 'ChromatinAssay'
granges(x, use.names = TRUE, use.mcols = FALSE, ...)
## S4 method for signature 'Seurat'
granges(x, use.names = TRUE, use.mcols = FALSE, ...)
```

GRangesToString 69

#### **Arguments**

x A ChromatinAssay object

use.names Whether the names on the genomic ranges should be propagated to the returned

object.

use.mcols Not supported for ChromatinAssay objects

... Additional arguments

### Value

Returns a GRanges object

#### **Functions**

• granges(Seurat): method for Seurat objects

#### See Also

- granges in the GenomicRanges package.
- ChromatinAssay-class

### **Examples**

```
granges(atac_small)
```

GRangesToString

GRanges to String

# Description

Convert GRanges object to a vector of strings

### Usage

```
GRangesToString(grange, sep = c("-", "-"))
```

# Arguments

grange A GRanges object

sep Vector of separators to use for genomic string. First element is used to separate

chromosome and coordinates, second separator is used to separate start and end

coordinates.

### Value

Returns a character vector

70 Identify Variants

#### **Examples**

```
GRangesToString(grange = blacklist_hg19)
```

head.Fragment

Return the first rows of a fragment file

# Description

Returns the first n rows of a fragment file. This allows the content of a fragment file to be inspected.

### Usage

```
## S3 method for class 'Fragment' head(x, n = 6L, ...)
```

### **Arguments**

x a Fragment object
 n an integer specifying the number of rows to return from the fragment file
 ... additional arguments passed to read.table

#### Value

The first n rows of a fragment file as a data. frame with the following columns: chrom, start, end, barcode, readCount.

IdentifyVariants

Identify mitochondrial variants

# Description

Identify mitochondrial variants present in single cells.

### Usage

```
IdentifyVariants(object, ...)
## Default S3 method:
IdentifyVariants(
  object,
  refallele,
  stabilize_variance = TRUE,
  low_coverage_threshold = 10,
  verbose = TRUE,
  ...
```

InsertionBias 71

```
## S3 method for class 'Assay'
IdentifyVariants(object, refallele, ...)
## S3 method for class 'StdAssay'
IdentifyVariants(object, refallele, ...)
## S3 method for class 'Seurat'
IdentifyVariants(object, refallele, assay = NULL, ...)
```

### Arguments

object A Seurat object

... Arguments passed to other methods

refallele A dataframe containing reference alleles for the mitochondrial genome.

stabilize\_variance

Stabilize variance

low\_coverage\_threshold

Low coverage threshold

verbose Display messages

assay Name of assay to use. If NULL, use the default assay.

#### Value

Returns a dataframe

### **Examples**

```
## Not run:
data.dir <- "path/to/data/directory"
mgatk <- ReadMGATK(dir = data.dir)
variant.df <- IdentifyVariants(
  object = mgatk$counts,
  refallele = mgatk$refallele
)
## End(Not run)</pre>
```

InsertionBias

Compute Tn5 insertion bias

### **Description**

Counts the Tn5 insertion frequency for each DNA hexamer.

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#### Usage

```
InsertionBias(object, ...)
## S3 method for class 'ChromatinAssay'
InsertionBias(object, genome, region = "chr1-1-249250621", verbose = TRUE, ...)
## S3 method for class 'Seurat'
InsertionBias(
   object,
   genome,
   assay = NULL,
   region = "chr1-1-249250621",
   verbose = TRUE,
   ...
)
```

### Arguments

object A Seurat or ChromatinAssay object
... Additional arguments passed to StringToGRanges
genome A BSgenome object or any other object supported by getSeq. Do showMethods("getSeq") to get the list of all supported object types.
region Genomic region to use when assessing bias.
verbose Display messages
assay Name of assay to use

#### Value

Returns a Seurat object

```
## Not run:
library(BSgenome.Mmusculus.UCSC.mm10)

region.use <- GRanges(
    seqnames = c('chr1', 'chr2'),
    IRanges(start = c(1,1), end = c(195471971, 182113224))
)

InsertionBias(
    object = atac_small,
    genome = BSgenome.Mmusculus.UCSC.mm10,
    region = region.use
)

## End(Not run)</pre>
```

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inter-range-methods

Inter-range transformations for ChromatinAssay objects

### **Description**

The range, reduce, gaps, disjoin, isDisjoint, disjointBins methods are available for ChromatinAssay objects.

```
## S4 method for signature 'ChromatinAssay'
range(x, ..., with.revmap = FALSE, na.rm = FALSE)
## S4 method for signature 'Seurat'
range(x, ..., with.revmap = FALSE, na.rm = FALSE)
## S4 method for signature 'ChromatinAssay'
reduce(x, drop.empty.ranges = FALSE, ...)
## S4 method for signature 'Seurat'
reduce(x, drop.empty.ranges = FALSE, ...)
## S4 method for signature 'ChromatinAssay'
gaps(x, start = NA, end = NA)
## S4 method for signature 'Seurat'
gaps(x, start = NA, end = NA)
## S4 method for signature 'ChromatinAssay'
disjoin(x, ...)
## S4 method for signature 'Seurat'
disjoin(x, ...)
## S4 method for signature 'ChromatinAssay'
isDisjoint(x, ...)
## S4 method for signature 'Seurat'
isDisjoint(x, ...)
## S4 method for signature 'ChromatinAssay'
disjointBins(x, ...)
## S4 method for signature 'Seurat'
disjointBins(x, ...)
```

74 IntersectMatrix

## Arguments

#### **Functions**

• range(Seurat): method for Seurat objects

• reduce(ChromatinAssay): method for ChromatinAssay objects

• reduce(Seurat): method for Seurat objects

• gaps(ChromatinAssay): method for ChromatinAssay objects

• gaps(Seurat): method for Seurat objects

• disjoin(ChromatinAssay): method for ChromatinAssay objects

• disjoin(Seurat): method for Seurat objects

• isDisjoint(ChromatinAssay): method for ChromatinAssay objects

• isDisjoint(Seurat): method for Seurat objects

• disjointBins(ChromatinAssay): method for ChromatinAssay objects

• disjointBins(Seurat): method for Seurat objects

### See Also

- inter-range-methods in the **IRanges** package.
- inter-range-methods in the GenomicRanges package
- ChromatinAssay-class

 ${\tt IntersectMatrix}$ 

Intersect genomic coordinates with matrix rows

### **Description**

Remove or retain matrix rows that intersect given genomic regions

Jaccard 75

### Usage

```
IntersectMatrix(
  matrix,
  regions,
  invert = FALSE,
  sep = c("-", "-"),
  verbose = TRUE,
  ...
)
```

### **Arguments**

matrix A matrix with genomic regions in the rows A set of genomic regions to intersect with regions in the matrix. Either a vector regions of strings encoding the genomic coordinates, or a GRanges object. invert Discard rows intersecting the genomic regions supplied, rather than retain. A length-2 character vector containing the separators to be used for extracting sep genomic coordinates from a string. The first element will be used to separate the chromosome name from coordinates, and the second element used to separate start and end coordinates. verbose Display messages Additional arguments passed to findOverlaps . . .

### Value

Returns a sparse matrix

### **Examples**

```
counts <- matrix(data = rep(0, 12), ncol = 2)
rownames(counts) <- c("chr1-565107-565550","chr1-569174-569639",
"chr1-713460-714823","chr1-752422-753038",
"chr1-762106-763359","chr1-779589-780271")
IntersectMatrix(matrix = counts, regions = blacklist_hg19)</pre>
```

Jaccard

Calculate the Jaccard index between two matrices

## Description

Finds the Jaccard similarity between rows of the two matrices. Note that the matrices must be binary, and any rows with zero total counts will result in an NaN entry that could cause problems in downstream analyses.

```
Jaccard(x, y)
```

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# Arguments

x The first matrix y The second matrix

#### **Details**

This will calculate the raw Jaccard index, without normalizing for the expected similarity between cells due to differences in sequencing depth.

#### Value

Returns a matrix

# Examples

```
x \leftarrow matrix(data = sample(c(0, 1), size = 25, replace = TRUE), ncol = 5)
Jaccard(x = x, y = x)
```

LinkPeaks

Link peaks to genes

## **Description**

Find peaks that are correlated with the expression of nearby genes. For each gene, this function computes the correlation coefficient between the gene expression and accessibility of each peak within a given distance from the gene TSS, and computes an expected correlation coefficient for each peak given the GC content, accessibility, and length of the peak. The expected coefficient values for the peak are then used to compute a z-score and p-value.

```
LinkPeaks(
  object,
  peak.assay,
  expression.assay,
  peak.slot = "counts",
  expression.slot = "data",
  method = "pearson",
  gene.coords = NULL,
  distance = 5e+05,
  min.distance = NULL,
  min.cells = 10,
  genes.use = NULL,
  n_{sample} = 200,
  pvalue_cutoff = 0.05,
  score\_cutoff = 0.05,
  gene.id = FALSE,
  verbose = TRUE
)
```

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#### **Arguments**

object A Seurat object

peak.assay Name of assay containing peak information

expression.assay

Name of assay containing gene expression information

peak.slot Name of slot to pull chromatin data from

expression.slot

Name of slot to pull expression data from

method Correlation method to use. One of "pearson" or "spearman"

gene.coords GRanges object containing coordinates of genes in the expression assay. If

NULL, extract from gene annotations stored in the assay.

distance Distance threshold for peaks to include in regression model

min.distance Minimum distance between peak and TSS to include in regression model. If

NULL (default), no minimum distance is used.

min.cells Minimum number of cells positive for the peak and gene needed to include in

the results.

genes.use Genes to test. If NULL, determine from expression assay.

n\_sample Number of peaks to sample at random when computing the null distribution.

pvalue\_cutoff Minimum p-value required to retain a link. Links with a p-value equal or greater

than this value will be removed from the output.

score\_cutoff Minimum absolute value correlation coefficient for a link to be retained

gene.id Set to TRUE if genes in the expression assay are named using gene IDs rather

than gene names.

verbose Display messages

#### **Details**

This function was inspired by the method originally described by SHARE-seq (Sai Ma et al. 2020, Cell). Please consider citing the original SHARE-seq work if using this function: doi:10.1016/j.cell.2020.09.056

#### Value

Returns a Seurat object with the Links information set. This is a granges object accessible via the Links function, with the following information:

- score: the correlation coefficient between the accessibility of the peak and expression of the gene
- zscore: the z-score of the correlation coefficient, computed based on the distribution of correlation coefficients from a set of background peaks
- pvalue: the p-value associated with the z-score for the link
- gene: name of the linked gene
- peak: name of the linked peak

78 LinkPlot

LinkPlot

Plot linked genomic elements

## **Description**

Display links between pairs of genomic elements within a given region of the genome.

### Usage

```
LinkPlot(
  object,
  region,
  assay = NULL,
  min.cutoff = 0,
  sep = c("-", "-"),
  extend.upstream = 0,
  extend.downstream = 0,
  scale.linewidth = FALSE
)
```

## Arguments

A Seurat object object A genomic region to plot region Name of assay to use. If NULL, use the default assay. assay min.cutoff Minimum absolute score for link to be plotted. Separators to use for strings encoding genomic coordinates. First element is sep used to separate the chromosome from the coordinates, second element is used to separate the start from end coordinate. extend.upstream Number of bases to extend the region upstream. extend.downstream Number of bases to extend the region downstream.

Scale thickness of the line according to link score.

# Value

Returns a ggplot object

scale.linewidth

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Links

Get or set links information

## **Description**

Get or set the genomic link information for a Seurat object or ChromatinAssay

# Usage

```
Links(object, ...)

Links(object, ...) <- value

## S3 method for class 'ChromatinAssay'
Links(object, ...)

## S3 method for class 'Seurat'
Links(object, ...)

## S3 replacement method for class 'ChromatinAssay'
Links(object, ...) <- value

## S3 replacement method for class 'Seurat'
Links(object, ...) <- value
```

## **Arguments**

```
object A Seurat object
... Arguments passed to other methods
value A GRanges object
```

# **Examples**

```
Links(atac_small[["peaks"]])
Links(atac_small)
links <- Links(atac_small)
Links(atac_small[["peaks"]]) <- links
links <- Links(atac_small)
Links(atac_small) <- links</pre>
```

80 MatchRegionStats

LookupGeneCoords

Get gene coordinates

# Description

Extract the coordinates of the longest transcript for a gene stored in the annotations within an object.

# Usage

```
LookupGeneCoords(object, gene, assay = NULL)
```

### **Arguments**

object A Seurat object

gene Name of a gene to extract

assay Name of assay to use

# **Examples**

```
LookupGeneCoords(atac_small, gene = "MIR1302-10")
```

 ${\tt MatchRegionStats}$ 

Match DNA sequence characteristics

# Description

Return a vector if genomic regions that match the distribution of a set of query regions for any given set of characteristics, specified in the input meta. feature dataframe.

```
MatchRegionStats(
  meta.feature,
  query.feature,
  features.match = c("GC.percent"),
  n = 10000,
  verbose = TRUE,
  ...
)
```

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### Arguments

meta. feature A dataframe containing DNA sequence information for features to choose from query. feature A dataframe containing DNA sequence information for features to match.

Mhich features of the query to match when selecting a set of regions. A vector of column names present in the feature metadata can be supplied to match multiple characteristics at once. Default is GC content.

Number of regions to select, with characteristics matching the query verbose

Display messages

Arguments passed to other functions

#### **Details**

For each requested feature to match, a density distribution is estimated using the density function, and a set of weights for each feature in the dataset estimated based on the density distribution. If multiple features are to be matched (for example, GC content and overall accessibility), a joint density distribution is then computed by multiplying the individual feature weights. A set of features with characteristics matching the query regions is then selected using the sample function, with the probability of randomly selecting each feature equal to the joint density distribution weight.

#### Value

Returns a character vector

### **Examples**

```
metafeatures <- SeuratObject::GetAssayData(
  object = atac_small[['peaks']], layer = 'meta.features')
query.feature <- metafeatures[1:10, ]
features.choose <- metafeatures[11:nrow(metafeatures), ]
MatchRegionStats(
  meta.feature = features.choose,
  query.feature = query.feature,
  features.match = "percentile",
  n = 10
)</pre>
```

Motif-class

The Motif class

### Description

The Motif class is designed to store DNA sequence motif information, including motif PWMs or PFMs, motif positions, and metadata.

82 MotifCounts

### Slots

data A sparse, binary, feature x motif matrix. Columns correspond to motif IDs, rows correspond to genomic features (peaks or bins). Entries in the matrix should be 1 if the genomic feature contains the motif, and 0 otherwise.

pwm A named list of position weight matrices

motif.names A list containing the name of each motif

positions A GRangesList object containing exact positions of each motif.

meta.data A dataframe for storage of additional information related to each motif. This could include the names of proteins that bind the motif.

MotifCounts

Count fragments surrounding motif sites

#### **Description**

Count the number of sequenced DNA fragments in a region surrounding each instance of a given DNA sequence motif.

## Usage

```
MotifCounts(
  object,
  motifs,
  flanking.region = 1000,
  assay = NULL,
  verbose = TRUE,
  ...
)
```

## Arguments

object A Seurat object

motifs A list of DNA sequence motif names. One matrix will be generated for each

motif

flanking.region

Amount of sequence to include surrounding the motif itself

assay Name of assay to use. Must be a ChromatinAssay

verbose Display messages

... Additional arguments passed to FeatureMatrix

### Value

Returns a list of sparse matrices

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MotifPlot

Plot DNA sequence motif

### **Description**

Plot position weight matrix or position frequency matrix for different DNA sequence motifs.

## Usage

```
MotifPlot(object, motifs, assay = NULL, use.names = TRUE, ...)
```

# Arguments

```
object A Seurat object
motifs A list of motif IDs or motif names to plot
assay Name of the assay to use
use.names Use motif names stored in the motif object
... Additional parameters passed to ggseqlogo
```

### Value

Returns a ggplot object

### **Examples**

```
motif.obj <- Motifs(atac_small)
MotifPlot(atac_small, motifs = head(colnames(motif.obj)))</pre>
```

Motifs

Get or set a motif information

### **Description**

Get or set the Motif object for a Seurat object or ChromatinAssay.

```
Motifs(object, ...)
Motifs(object, ...) <- value
## S3 method for class 'ChromatinAssay'
Motifs(object, ...)</pre>
```

```
## S3 method for class 'Seurat'
Motifs(object, ...)
## S3 replacement method for class 'ChromatinAssay'
Motifs(object, ...) <- value
## S3 replacement method for class 'Seurat'
Motifs(object, ...) <- value</pre>
```

#### Arguments

object A Seurat object

... Arguments passed to other methods

value A Motif object

## **Examples**

```
Motifs(atac_small[["peaks"]])
Motifs(atac_small)
motifs <- Motifs(atac_small)
Motifs(atac_small[["peaks"]]) <- motifs
motifs <- Motifs(atac_small)
Motifs(atac_small) <- motifs</pre>
```

nearest-methods

Find the nearest range neighbors for ChromatinAssay objects

## **Description**

The precede, follow, nearest, distance, distanceToNearest methods are available for ChromatinAssay objects.

```
## S4 method for signature 'ANY,ChromatinAssay'
precede(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ChromatinAssay,ANY'
precede(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ChromatinAssay,ChromatinAssay'
precede(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ANY,Seurat'
precede(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'Seurat,ANY'
```

```
precede(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'Seurat, Seurat'
precede(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ANY, ChromatinAssay'
follow(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ChromatinAssay, ANY'
follow(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ChromatinAssay, ChromatinAssay'
follow(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ANY, Seurat'
follow(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'Seurat, ANY'
follow(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'Seurat, Seurat'
follow(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ANY, ChromatinAssay'
nearest(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ChromatinAssay, ANY'
nearest(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ChromatinAssay, ChromatinAssay'
nearest(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ANY, Seurat'
nearest(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'Seurat, ANY'
nearest(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'Seurat, Seurat'
nearest(x, subject, select = c("arbitrary", "all"), ignore.strand = FALSE)
## S4 method for signature 'ANY, ChromatinAssay'
distance(x, y, ignore.strand = FALSE, ...)
## S4 method for signature 'ChromatinAssay, ANY'
distance(x, y, ignore.strand = FALSE, ...)
## S4 method for signature 'ChromatinAssay, ChromatinAssay'
```

```
distance(x, y, ignore.strand = FALSE, ...)
## S4 method for signature 'ANY, Seurat'
distance(x, y, ignore.strand = FALSE, ...)
## S4 method for signature 'Seurat, ANY'
distance(x, y, ignore.strand = FALSE, ...)
## S4 method for signature 'Seurat, Seurat'
distance(x, y, ignore.strand = FALSE, ...)
## S4 method for signature 'ANY, ChromatinAssay'
distanceToNearest(x, subject, ignore.strand = FALSE, ...)
## S4 method for signature 'ChromatinAssay, ANY'
distanceToNearest(x, subject, ignore.strand = FALSE, ...)
## S4 method for signature 'ChromatinAssay, ChromatinAssay'
distanceToNearest(x, subject, ignore.strand = FALSE, ...)
## S4 method for signature 'ANY, Seurat'
distanceToNearest(x, subject, ignore.strand = FALSE, ...)
## S4 method for signature 'Seurat, ANY'
distanceToNearest(x, subject, ignore.strand = FALSE, ...)
## S4 method for signature 'Seurat, Seurat'
distanceToNearest(x, subject, ignore.strand = FALSE, ...)
```

### **Arguments**

X	A query ChromatinAssay object
subject	The subject GRanges or ChromatinAssay object. If missing, $\boldsymbol{x}$ is used as the subject.
select	Logic for handling ties. See ${\sf nearest-methods}$ in the ${\sf GenomicRanges}$ package.
ignore.strand	Logical argument controlling whether strand information should be ignored.
у	For the distance method, a GRanges object or a ChromatinAssay object
	Additional arguments for methods

## **Functions**

- precede(x = ChromatinAssay, subject = ANY): method for ChromatinAssay, ANY
- precede(x = ChromatinAssay, subject = ChromatinAssay): method for ChromatinAssay, ChromatinAssay
- precede(x = ANY, subject = Seurat): method for ANY, Seurat
- precede(x = Seurat, subject = ANY): method for Seurat, ANY

- precede(x = Seurat, subject = Seurat): method for Seurat, Seurat
- follow(x = ANY, subject = ChromatinAssay): method for ANY, ChromatinAssay
- follow(x = ChromatinAssay, subject = ANY): method for ChromatinAssay, ANY
- follow(x = ChromatinAssay, subject = ChromatinAssay): method for ChromatinAssay, ChromatinAssay
- follow(x = ANY, subject = Seurat): method for ANY, Seurat
- follow(x = Seurat, subject = ANY): method for Seurat, ANY
- follow(x = Seurat, subject = Seurat): method for Seurat, Seurat
- nearest(x = ANY, subject = ChromatinAssay): method for ANY, ChromatinAssay
- nearest(x = ChromatinAssay, subject = ANY): method for ChromatinAssay, ANY
- nearest(x = ChromatinAssay, subject = ChromatinAssay): method for ChromatinAssay, ChromatinAssay
- nearest(x = ANY, subject = Seurat): method for ANY, Seurat
- nearest(x = Seurat, subject = ANY): method for Seurat, ANY
- nearest(x = Seurat, subject = Seurat): method for Seurat, Seurat
- distance(x = ANY, y = ChromatinAssay): method for ANY, ChromatinAssay
- distance(x = ChromatinAssay, y = ANY): method for ChromatinAssay, ANY
- distance(x = ChromatinAssay, y = ChromatinAssay): method for ChromatinAssay, ChromatinAssay
- distance(x = ANY, y = Seurat): method for ANY, Seurat
- distance(x = Seurat, y = ANY): method for Seurat, ANY
- distance(x = Seurat, y = Seurat): method for Seurat, Seurat
- distanceToNearest(x = ANY, subject = ChromatinAssay): method for ANY, ChromatinAssay
- distanceToNearest(x = ChromatinAssay, subject = ANY): method for ChromatinAssay, ANY
- distanceToNearest(x = ChromatinAssay, subject = ChromatinAssay): method for ChromatinAssay, ChromatinAssay
- distanceToNearest(x = ANY, subject = Seurat): method for ANY, Seurat
- distanceToNearest(x = Seurat, subject = ANY): method for Seurat, ANY
- distanceToNearest(x = Seurat, subject = Seurat): method for Seurat, Seurat

#### See Also

- nearest-methods in the **IRanges** package.
- nearest-methods in the GenomicRanges package
- ChromatinAssay-class

88 NucleosomeSignal

NucleosomeSignal	NucleosomeSignal
------------------	------------------

# Description

Calculate the strength of the nucleosome signal per cell. Computes the ratio of fragments between 147 bp and 294 bp (mononucleosome) to fragments < 147 bp (nucleosome-free)

# Usage

```
NucleosomeSignal(
  object,
  assay = NULL,
  n = ncol(object) * 5000,
  verbose = TRUE,
  ...
)
```

# Arguments

object	A Seurat object
assay	Name of assay to use. Only required if a fragment path is not provided. If NULL, use the active assay.
n	Number of lines to read from the fragment file. If NULL, read all lines. Default scales with the number of cells in the object.
verbose	Display messages
	Arguments passed to other functions

## Value

Returns a Seurat object with added metadata for the ratio of mononucleosomal to nucleosome-free fragments per cell, and the percentile rank of each ratio.

# **Examples**

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
Fragments(atac_small) <- CreateFragmentObject(
  path = fpath,
  cells = colnames(atac_small),
  tolerance = 0.5
)
NucleosomeSignal(object = atac_small)</pre>
```

PeakPlot 89

PeakPlot	Plot peaks in a genomic region	
----------	--------------------------------	--

# Description

Display the genomic ranges in a ChromatinAssay object that fall in a given genomic region

# Usage

```
PeakPlot(
  object,
  region,
  assay = NULL,
  peaks = NULL,
  group.by = NULL,
  color = "dimgrey",
  sep = c("-", "-"),
  extend.upstream = 0,
  extend.downstream = 0)
```

# Arguments

object	A Seurat object	
region	A genomic region to plot	
assay	Name of assay to use. If NULL, use the default assay.	
peaks	A GRanges object containing peak coordinates. If NULL, use coordinates stored in the Seurat object.	
group.by	Name of variable in feature metadata (if using ranges in the Seurat object) or genomic ranges metadata (if using supplied ranges) to color ranges by. If NULL, do not color by any metadata variable.	
color	Color to use. If group.by is not NULL, this can be a custom color scale (see examples).	
sep	Separators to use for strings encoding genomic coordinates. First element is used to separate the chromosome from the coordinates, second element is used to separate the start from end coordinate.	
extend.upstream		
	Number of bases to extend the region upstream.	
extend.downstream		
	Number of bases to extend the region downstream.	

# Value

Returns a ggplot object

90 PlotFootprint

## **Examples**

```
# plot peaks in assay
PeakPlot(atac_small, region = "chr1-710000-715000")

# manually set color
PeakPlot(atac_small, region = "chr1-710000-715000", color = "red")

# color by a variable in the feature metadata
PeakPlot(atac_small, region = "chr1-710000-715000", group.by = "count")
```

PlotFootprint

Plot motif footprinting results

# Description

Plot motif footprinting results

## Usage

```
PlotFootprint(
  object,
  features,
  assay = NULL,
  group.by = NULL,
  split.by = NULL,
  idents = NULL,
  label = TRUE,
  repel = TRUE,
  show.expected = TRUE,
  normalization = "subtract",
  label.top = 3,
  label.idents = NULL
)
```

# Arguments

object	A Seurat object
features	A vector of features to plot
assay	Name of assay to use
group.by	A grouping variable
split.by	A metadata variable to split the plot by. For example, grouping by "celltype" and splitting by "batch" will create separate plots for each celltype and batch.
idents	Set of identities to include in the plot
label	TRUE/FALSE value to control whether groups are labeled.

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repel	Repel labels from each other
show.expected	Plot the expected Tn5 integration frequency below the main footprint plot
normalization	Method to normalize for Tn5 DNA sequence bias. Options are "subtract", "divide", or NULL to perform no bias correction.
label.top	Number of groups to label based on highest accessibility in motif flanking region.
label.idents	Vector of identities to label. If supplied, label.top will be ignored.

ReadMGATK Read MGATK output

# Description

Read output files from MGATK (https://github.com/caleblareau/mgatk).

## Usage

```
ReadMGATK(dir, verbose = TRUE)
```

## Arguments

dir Path to directory containing MGATK output files

verbose Display messages

#### Value

Returns a list containing a sparse matrix (counts) and two dataframes (depth and refallele).

The sparse matrix contains read counts for each base at each position and strand.

The depth dataframe contains the total depth for each cell. The refallele dataframe contains the reference genome allele at each position.

## **Examples**

```
## Not run:
data.dir <- system.file("extdata", "test_mgatk", package="Signac")
mgatk <- ReadMGATK(dir = data.dir)
## End(Not run)</pre>
```

92 RegionHeatmap

RegionHeatmap Region heatmap

# Description

Plot fragment counts within a set of regions.

# Usage

```
RegionHeatmap(
  object,
  key,
  assay = NULL,
  idents = NULL,
  normalize = TRUE,
  upstream = 3000,
  downstream = 3000,
  max.cutoff = "q95",
  cols = NULL,
  min.counts = 1,
  window = (upstream + downstream)/30,
  order = TRUE,
  nrow = NULL
)
```

# Arguments

object	A Seurat object
key	Name of key to pull data from. Stores the results from RegionMatrix
assay	Name of assay to use. If a list or vector of assay names is given, data will be plotted from each assay. Note that all assays must contain RegionMatrix results with the same key. Sorting will be defined by the first assay in the list
idents	Cell identities to include. Note that cells cannot be regrouped, this will require re-running RegionMatrix to generate a new set of matrices
normalize	Normalize by number of cells in each group
upstream	Number of bases to include upstream of region. If NULL, use all bases that were included in the RegionMatrix function call. Note that this value cannot be larger than the value for upstream given in the original RegionMatrix function call. If NULL, use parameters that were given in the RegionMatrix function call
downstream	Number of bases to include downstream of region. See documentation for upstream
max.cutoff	Maximum cutoff value. Data above this value will be clipped to the maximum value. A quantile maximum can be specified in the form of "q##" where "##" is the quantile (eg, "q90" for 90th quantile). If NULL, no cutoff will be set

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vector of colors to use as the maximum value of the color scale. One color must

be supplied for each assay. If NULL, the default ggplot2 colors are used.

min. counts Minimum total counts to display region in plot

window Smoothing window to apply

order Order regions by the total number of fragments in the region across all included

identities

nrow Number of rows to use when creating plot. If NULL, chosen automatically by

ggplot2

### Value

Returns a ggplot2 object

#### See Also

RegionMatrix

 ${\sf RegionMatrix}$ 

Region enrichment analysis

## Description

Count fragments within a set of regions for different groups of cells.

```
RegionMatrix(object, ...)
## S3 method for class 'Seurat'
RegionMatrix(
  object,
  regions,
  key,
  assay = NULL,
  group.by = NULL,
  idents = NULL,
  upstream = 3000,
  downstream = 3000,
  verbose = TRUE,
)
## S3 method for class 'ChromatinAssay'
RegionMatrix(
  object,
  regions,
```

94 RegionMatrix

```
key,
 assay = NULL,
 group.by = NULL,
 idents = NULL,
 upstream = 3000,
 downstream = 3000,
 verbose = TRUE,
)
## Default S3 method:
RegionMatrix(
 object,
 regions,
 key,
 assay = NULL,
 group.by = NULL,
 idents = NULL,
 upstream = 3000,
 downstream = 3000,
 verbose = TRUE,
)
```

# Arguments

object	A Seurat or ChromatinAssay object
	Arguments passed to other methods
regions	A GRanges object containing the set of genomic ranges to quantify
key	Name to store resulting matrices under
assay	Name of assay to use. If NULL, use the default assay
group.by	Grouping variable to use when aggregating data across cells. If NULL, use the active cell identities
idents	Cell identities to include. If NULL, include all identities
upstream	Number of bases to extend regions upstream
downstream	Number of bases to extend regions downstream
verbose	Display messages

# Value

Returns a Seurat object

RegionPlot 95

RegionPlot Region plot
------------------------

# Description

Plot fragment counts within a set of regions.

# Usage

```
RegionPlot(
  object,
  key,
  assay = NULL,
  idents = NULL,
  normalize = TRUE,
  upstream = NULL,
  downstream = NULL,
  window = (upstream + downstream)/500,
  nrow = NULL
)
```

# Arguments

object	A Seurat object
key	Name of key to pull data from. Stores the results from RegionMatrix
assay	Name of assay to use. If a list or vector of assay names is given, data will be plotted from each assay. Note that all assays must contain RegionMatrix results with the same key. Sorting will be defined by the first assay in the list
idents	Cell identities to include. Note that cells cannot be regrouped, this will require re-running RegionMatrix to generate a new set of matrices
normalize	Normalize by number of cells in each group
upstream	Number of bases to include upstream of region. If NULL, use all bases that were included in the RegionMatrix function call. Note that this value cannot be larger than the value for upstream given in the original RegionMatrix function call. If NULL, use parameters that were given in the RegionMatrix function call
downstream	Number of bases to include downstream of region. See documentation for upstream
window	Smoothing window to apply
nrow	Number of rows to use when creating plot. If NULL, chosen automatically by ggplot2

## Value

Returns a ggplot2 object

96 RegionStats

### See Also

RegionMatrix

RegionStats Compute base composition information for genomic ranges

# Description

Compute the GC content, region lengths, and dinucleotide base frequencies for regions in the assay and add to the feature metadata.

### Usage

```
RegionStats(object, ...)
## Default S3 method:
RegionStats(object, genome, verbose = TRUE, ...)
## S3 method for class 'ChromatinAssay'
RegionStats(object, genome, verbose = TRUE, ...)
## S3 method for class 'Seurat'
RegionStats(object, genome, assay = NULL, verbose = TRUE, ...)
```

## **Arguments**

object A Seurat object, Assay object, or set of genomic ranges

... Arguments passed to other methods

genome A BSgenome object or any other object supported by getSeq. Do showMethods("getSeq")

to get the list of all supported object types.

verbose Display messages assay Name of assay to use

### Value

Returns a dataframe

## **Examples**

```
## Not run:
library(BSgenome.Hsapiens.UCSC.hg19)
RegionStats(
  object = rownames(atac_small),
   genome = BSgenome.Hsapiens.UCSC.hg19
)
```

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```
## End(Not run)
## Not run:
library(BSgenome.Hsapiens.UCSC.hg19)
RegionStats(
   object = atac_small[['peaks']],
   genome = BSgenome.Hsapiens.UCSC.hg19
)

## End(Not run)
## Not run:
library(BSgenome.Hsapiens.UCSC.hg19)
RegionStats(
   object = atac_small,
   assay = 'bins',
   genome = BSgenome.Hsapiens.UCSC.hg19
)

## End(Not run)
```

RunChromVAR

Run chromVAR

# Description

Wrapper to run chromVAR on an assay with a motif object present. Will return a new Seurat assay with the motif activities (the deviations in chromatin accessibility across the set of regions) as a new assay.

## Usage

```
RunChromVAR(object, ...)
## S3 method for class 'ChromatinAssay'
RunChromVAR(object, genome, motif.matrix = NULL, verbose = TRUE, ...)
## S3 method for class 'Seurat'
RunChromVAR(
  object,
  genome,
  motif.matrix = NULL,
  assay = NULL,
  new.assay.name = "chromvar",
  ...
)
```

## **Arguments**

object

A Seurat object

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... Additional arguments passed to getBackgroundPeaks

genome A BSgenome object or string stating the genome build recognized by getBSgenome.

Motif.matrix A peak x motif matrix. If NULL, pull the peak x motif matrix from a Motif

object stored in the assay.

verbose Display messages assay Name of assay to use

new.assay.name Name of new assay used to store the chromVAR results. Default is "chromvar".

#### **Details**

See the chromVAR documentation for more information: https://greenleaflab.github.io/chromVAR/index.html

See the chromVAR paper: https://www.nature.com/articles/nmeth.4401

#### Value

Returns a Seurat object with a new assay

## **Examples**

```
## Not run:
library(BSgenome.Hsapiens.UCSC.hg19)
RunChromVAR(object = atac_small[["peaks"]], genome = BSgenome.Hsapiens.UCSC.hg19)
## End(Not run)
## Not run:
library(BSgenome.Hsapiens.UCSC.hg19)
RunChromVAR(object = atac_small, genome = BSgenome.Hsapiens.UCSC.hg19)
## End(Not run)
```

RunSVD

Run singular value decomposition

### **Description**

Run partial singular value decomposition using irlba

```
RunSVD(object, ...)
## Default S3 method:
RunSVD(
  object,
  assay = NULL,
  n = 50,
```

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```
scale.embeddings = TRUE,
  reduction.key = "LSI_",
  scale.max = NULL,
  verbose = TRUE,
  irlba.work = n * 3,
  tol = 1e-05,
)
## S3 method for class 'Assay'
RunSVD(
  object,
  assay = NULL,
  features = NULL,
  n = 50,
  reduction.key = "LSI_",
  scale.max = NULL,
  verbose = TRUE,
)
## S3 method for class 'StdAssay'
RunSVD(
 object,
 assay = NULL,
  features = NULL,
 n = 50,
  reduction.key = "LSI_",
  scale.max = NULL,
 verbose = TRUE,
  . . .
)
## S3 method for class 'Seurat'
RunSVD(
 object,
  assay = NULL,
  features = NULL,
  n = 50,
  reduction.key = "LSI_",
  reduction.name = "lsi",
  scale.max = NULL,
  verbose = TRUE,
)
```

## **Arguments**

object A Seurat object

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... Arguments passed to other methods

assay Which assay to use. If NULL, use the default assay

n Number of singular values to compute

scale.embeddings

Scale cell embeddings within each component to mean 0 and SD 1 (default

TRUE).

reduction.key Key for dimension reduction object

scale.max Clipping value for cell embeddings. Default (NULL) is no clipping.

verbose Print messages

irlba.work work parameter for irlba. Working subspace dimension, larger values can

speed convergence at the cost of more memory use.

tol Tolerance (tol) parameter for irlba. Larger values speed up convergence due to

greater amount of allowed error.

features Which features to use. If NULL, use variable features reduction.name Name for stored dimension reduction object. Default 'svd'

#### Value

Returns a Seurat object

# Examples

```
x <- matrix(data = rnorm(100), ncol = 10)
RunSVD(x)
## Not run:
RunSVD(atac_small[['peaks']])
## End(Not run)
## Not run:
RunSVD(atac_small[['peaks']])
## End(Not run)
## Not run:
RunSVD(atac_small)
## End(Not run)</pre>
```

RunTFIDF

Compute the term-frequency inverse-document-frequency

## Description

Run term frequency inverse document frequency (TF-IDF) normalization on a matrix.

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```
RunTFIDF(object, ...)
## Default S3 method:
RunTFIDF(
  object,
  assay = NULL,
  method = 1,
  scale.factor = 10000,
  idf = NULL,
  verbose = TRUE,
)
## S3 method for class 'Assay'
RunTFIDF(
  object,
  assay = NULL,
  method = 1,
  scale.factor = 10000,
  idf = NULL,
  verbose = TRUE,
)
## S3 method for class 'StdAssay'
RunTFIDF(
  object,
  assay = NULL,
  method = 1,
  scale.factor = 10000,
  idf = NULL,
  verbose = TRUE,
)
## S3 method for class 'Seurat'
RunTFIDF(
  object,
  assay = NULL,
  method = 1,
  scale.factor = 10000,
  idf = NULL,
  verbose = TRUE,
)
```

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## **Arguments**

object	A Seurat object
	Arguments passed to other methods
assay	Name of assay to use
method	Which TF-IDF implementation to use. Choice of:
	• 1: The TF-IDF implementation used by Stuart & Butler et al. 2019 (doi:10.1101/460147). This computes $\log(TF \times IDF)$ .
	• 2: The TF-IDF implementation used by Cusanovich & Hill et al. 2018 (doi:10.1016/j.cell.2018.06.052). This computes $TF \times (\log(IDF))$ .
	• 3: The log-TF method used by Andrew Hill. This computes $\log(TF) \times \log(IDF)$ .
	• 4: The 10x Genomics method (no TF normalization). This computes $IDF$ .
scale.factor	Which scale factor to use. Default is 10000.
idf	A precomputed IDF vector to use. If NULL, compute based on the input data matrix.
verbose	Print progress

## **Details**

Four different TF-IDF methods are implemented. We recommend using method 1 (the default).

## Value

Returns a Seurat object

# References

https://en.wikipedia.org/wiki/Latent\_semantic\_analysis#Latent\_semantic\_indexing

# Examples

```
mat <- matrix(data = rbinom(n = 25, size = 5, prob = 0.2), nrow = 5)
RunTFIDF(object = mat)
RunTFIDF(atac_small[['peaks']])
RunTFIDF(atac_small[['peaks']])
RunTFIDF(object = atac_small)</pre>
```

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seqinfo-methods

Access and modify sequence information for ChromatinAssay objects

### **Description**

Methods for accessing and modifying Seqinfo object information stored in a ChromatinAssay object.

```
## S4 method for signature 'ChromatinAssay'
seqinfo(x)
## S4 replacement method for signature 'ChromatinAssay'
seqinfo(x) \leftarrow value
## S4 method for signature 'ChromatinAssay'
seqlevels(x)
## S4 replacement method for signature 'ChromatinAssay'
seqlevels(x) <- value</pre>
## S4 method for signature 'ChromatinAssay'
seqnames(x)
## S4 replacement method for signature 'ChromatinAssay'
seqnames(x) \leftarrow value
## S4 method for signature 'ChromatinAssay'
seglengths(x)
## S4 replacement method for signature 'ChromatinAssay'
seqlengths(x) \leftarrow value
## S4 method for signature 'ChromatinAssay'
genome(x)
## S4 replacement method for signature 'ChromatinAssay'
genome(x) <- value</pre>
## S4 method for signature 'ChromatinAssay'
isCircular(x)
## S4 replacement method for signature 'ChromatinAssay'
isCircular(x) <- value
## S4 method for signature 'Seurat'
```

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```
seqinfo(x)
## S4 replacement method for signature 'Seurat'
seqinfo(x) <- value</pre>
## S4 method for signature 'Seurat'
seglevels(x)
## S4 replacement method for signature 'Seurat'
seqlevels(x) \leftarrow value
## S4 method for signature 'Seurat'
seqnames(x)
## S4 replacement method for signature 'Seurat'
seqnames(x) \leftarrow value
## S4 method for signature 'Seurat'
seqlengths(x)
## S4 replacement method for signature 'Seurat'
seqlengths(x) \leftarrow value
## S4 method for signature 'Seurat'
genome(x)
## S4 replacement method for signature 'Seurat'
genome(x) <- value
## S4 method for signature 'Seurat'
isCircular(x)
## S4 replacement method for signature 'Seurat'
isCircular(x) <- value</pre>
```

## Arguments

x A ChromatinAssay object

value A Seqinfo object or name of a UCSC genome to store in the ChromatinAssay

#### **Functions**

- seqinfo(ChromatinAssay) <- value: set method for ChromatinAssay objects
- seqlevels(ChromatinAssay): get method for ChromatinAssay objects
- seqlevels(ChromatinAssay) <- value: set method for ChromatinAssay objects
- seqnames(ChromatinAssay): get method for ChromatinAssay objects
- seqnames(ChromatinAssay) <- value: set method for ChromatinAssay objects

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- seqlengths(ChromatinAssay): get method for ChromatinAssay objects
- seqlengths(ChromatinAssay) <- value: set method for ChromatinAssay objects
- genome(ChromatinAssay): get method for ChromatinAssay objects
- genome(ChromatinAssay) <- value: set method for ChromatinAssay objects
- isCircular(ChromatinAssay): get method for ChromatinAssay objects
- isCircular(ChromatinAssay) <- value: set method for ChromatinAssay objects
- seqinfo(Seurat): get method for Seurat objects
- seqinfo(Seurat) <- value: set method for Seurat objects
- seqlevels(Seurat): get method for Seurat objects
- seqlevels(Seurat) <- value: set method for Seurat objects
- seqnames(Seurat): get method for Seurat objects
- segnames(Seurat) <- value: set method for Seurat objects
- seqlengths(Seurat): get method for Seurat objects
- seqlengths(Seurat) <- value: set method for Seurat objects
- genome(Seurat): get method for Seurat objects
- genome(Seurat) <- value: set method for Seurat objects
- isCircular(Seurat): get method for Seurat objects
- isCircular(Seurat) <- value: set method for Seurat objects

### See Also

- seginfo in the **GenomeInfoDb** package.
- ChromatinAssay-class

SetMotifData

Set motif data

## Description

Set motif matrix for given assay

```
SetMotifData(object, ...)
## S3 method for class 'Motif'
SetMotifData(object, slot, new.data, ...)
## S3 method for class 'ChromatinAssay'
SetMotifData(object, slot, new.data, ...)
## S3 method for class 'Seurat'
SetMotifData(object, assay = NULL, ...)
```

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## **Arguments**

object	A Seurat object
	Arguments passed to other methods
slot	Name of slot to use
new.data	motif matrix to add. Should be matrix or sparse matrix class
assay	Name of assay whose data should be set

#### Value

Returns a Seurat object

## **Examples**

```
motif.obj <- SeuratObject::GetAssayData(
   object = atac_small[['peaks']], slot = "motifs"
)
SetMotifData(object = motif.obj, slot = 'data', new.data = matrix(1:2))
SetMotifData(
   object = atac_small[['peaks']], slot = 'data', new.data = matrix(1:2)
)
motif.matrix <- GetMotifData(object = atac_small)
SetMotifData(
object = atac_small, assay = 'peaks', slot = 'data', new.data = motif.matrix
)</pre>
```

SortIdents

Sorts cell metadata variable by similarity using hierarchical clustering

## **Description**

Compute distance matrix from a feature/variable matrix and perform hierarchical clustering to order variables (for example, cell types) according to their similarity.

```
SortIdents(
  object,
  layer = "data",
  assay = NULL,
  label = NULL,
  dendrogram = FALSE,
  method = "euclidean",
  verbose = TRUE
)
```

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## Arguments

object	A Seurat object containing single-cell data.
layer	The layer of the data to use (default is "data").
assay	Name of assay to use. If NULL, use the default assay
label	Metadata attribute to sort. If NULL, uses the active identities.
dendrogram	Logical, whether to plot the dendrogram (default is FALSE).
method	The distance method to use for hierarchical clustering (default is 'euclidean', other options from dist are 'maximum', 'manhattan', 'canberra', 'binary' and 'minkowski').

# verbose Display messages

#### Value

The Seurat object with metadata variable reordered by similarity. If the metadata variable was a character vector, it will be converted to a factor and the factor levels set according to the similarity ordering. If active identities were used (label=NULL), the levels will be updated according to similarity ordering.

### **Examples**

```
atac_small$test <- sample(1:10, ncol(atac_small), replace = TRUE)
atac_small <- SortIdents(object = atac_small, label = 'test')
print(levels(atac_small$test))</pre>
```

SplitFragments

Split fragment file by cell identities

### **Description**

Splits a fragment file into separate files for each group of cells. If splitting multiple fragment files containing common cell types, fragments originating from different files will be appended to the same file for one group of cell identities.

```
SplitFragments(
  object,
  assay = NULL,
  group.by = NULL,
  idents = NULL,
  outdir = getwd(),
  file.suffix = "",
  append = TRUE,
  buffer_length = 256L,
  verbose = TRUE
)
```

108 StringToGRanges

### **Arguments**

object A Seurat object
assay Name of assay to use

group.by Name of grouping variable to group cells by

idents List of identities to include outdir Directory to write output files

file.suffix Suffix to add to all file names (before file extension). If splitting multiple frag-

ment files without the append option set to TRUE, an additional numeric suffix

will be added to each file (eg, .1, .2).

append If splitting multiple fragment files, append cells from the same group (eg cluster)

to the same file. Note that this can cause the output file to be unsorted.

buffer\_length Size of buffer to be read from the fragment file. This must be longer than the

longest line in the file.

verbose Display messages

StringToGRanges String to GRanges

## Description

Convert a genomic coordinate string to a GRanges object

### Usage

```
StringToGRanges(regions, sep = c("-", "-"), ...)
```

## **Arguments**

regions Vector of genomic region strings

sep Vector of separators to use for genomic string. First element is used to separate

chromosome and coordinates, second separator is used to separate start and end

coordinates.

... Additional arguments passed to makeGRangesFromDataFrame

#### Value

Returns a GRanges object

### **Examples**

```
regions <- c('chr1-1-10', 'chr2-12-3121')
StringToGRanges(regions = regions)</pre>
```

subset.Fragment 109

subset.Fragment

Subset a Fragment object

# **Description**

Returns a subset of a Fragment-class object.

## Usage

```
## S3 method for class 'Fragment'
subset(x, cells = NULL, ...)
```

#### **Arguments**

x A Fragment objectcells Vector of cells to retain

... Arguments passed to other methods

#### Value

Returns a subsetted Fragment object

#### See Also

subset

# **Examples**

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
cells <- colnames(x = atac_small)
names(x = cells) <- paste0("test_", cells)
frags <- CreateFragmentObject(path = fpath, cells = cells, verbose = FALSE, tolerance = 0.5)
subset(frags, head(names(cells)))</pre>
```

subset.Motif

Subset a Motif object

# **Description**

Returns a subset of a Motif-class object.

# Usage

```
## S3 method for class 'Motif'
subset(x, features = NULL, motifs = NULL, ...)
## S3 method for class 'Motif'
x[i, j, ...]
```

110 SubsetMatrix

# **Arguments**

X	A Motif object
features	Which features to retain
motifs	Which motifs to retain
	Arguments passed to other methods
i	Which columns to retain
j	Which rows to retain

# Value

Returns a subsetted Motif object

#### See Also

subset

# **Examples**

```
motif.obj <- SeuratObject::GetAssayData(
  object = atac_small[['peaks']], layer = "motifs"
)
subset(x = motif.obj, features = head(rownames(motif.obj), 10))
motif.obj <- SeuratObject::GetAssayData(
  object = atac_small, assay = 'peaks', layer = 'motifs'
)
motif.obj[1:10,1:10]</pre>
```

SubsetMatrix

Subset matrix rows and columns

# **Description**

Subset the rows and columns of a matrix by removing rows and columns with less than the specified number of non-zero elements.

# Usage

```
SubsetMatrix(
  mat,
  min.rows = 1,
  min.cols = 1,
  max.row.val = 10,
  max.col.val = NULL
)
```

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# Arguments

mat	A matrix
min.rows	Minimum number of non-zero elements for the row to be retained
min.cols	Minimum number of non-zero elements for the column to be retained
max.row.val	Maximum allowed value in a row for the row to be retained. If NULL, don't set any limit.
max.col.val	Maximum allowed value in a column for the column to be retained. If NULL, don't set any limit.

#### Value

Returns a matrix

# **Examples**

```
SubsetMatrix(mat = volcano)
```

theme_browser	Genome browser theme

# Description

Theme applied to panels in the CoveragePlot function.

# Usage

```
theme_browser(..., legend = TRUE, axis.text.y = FALSE)
```

# **Arguments**

```
... Additional arguments
legend Display plot legend
axis.text.y Display y-axis text
```

# Examples

```
PeakPlot(atac_small, region = "chr1-710000-715000") + theme_browser()
```

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TilePlot

Plot integration sites per cell

#### **Description**

Plots the presence/absence of Tn5 integration sites for each cell within a genomic region.

# Usage

```
TilePlot(
  object,
  region,
  sep = c("-", "-"),
  tile.size = 100,
  tile.cells = 100,
  extend.upstream = 0,
  extend.downstream = 0,
  assay = NULL,
  cells = NULL,
  group.by = NULL,
  order.by = "total",
  idents = NULL)
```

#### Arguments

object	A Seurat object	
region	A set of genomic coordinates to show.	Can be a GRange

A set of genomic coordinates to show. Can be a GRanges object, a string encoding a genomic position, a gene name, or a vector of strings describing the genomic coordinates or gene names to plot. If a gene name is supplied, annota-

tions must be present in the assay.

sep Separators to use for strings encoding genomic coordinates. First element is

used to separate the chromosome from the coordinates, second element is used

to separate the start from end coordinate.

tile.size Size of the sliding window for per-cell fragment tile plot

tile.cells Number of cells to display fragment information for in tile plot.

 ${\tt extend.upstream}$ 

Number of bases to extend the region upstream.

extend.downstream

Number of bases to extend the region downstream.

assay Name of assay to use

cells Which cells to plot. Default all cells

group.by Name of grouping variable to group cells by. If NULL, use the current cell

identities

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order.by Option for determining how cells are chosen from each group. Options are

"total" or "random". "total" will select the top cells based on total number of

fragments in the region, "random" will select randomly.

idents List of cell identities to include in the plot. If NULL, use all identities.

#### Value

Returns a ggplot object

### **Examples**

```
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
fragments <- CreateFragmentObject(
  path = fpath,
  cells = colnames(atac_small),
  validate.fragments = FALSE
)
Fragments(atac_small) <- fragments
TilePlot(object = atac_small, region = c("chr1-713500-714500"))</pre>
```

**TSSEnrichment** 

Compute TSS enrichment score per cell

#### **Description**

Compute the transcription start site (TSS) enrichment score for each cell, as defined by ENCODE: https://www.encodeproject.org/data-standards/terms/.

# Usage

```
TSSEnrichment(
  object,
  tss.positions = NULL,
  n = NULL,
  fast = TRUE,
  assay = NULL,
  cells = NULL,
  process_n = 2000,
  verbose = TRUE,
  region_extension = 1000
)
```

#### **Arguments**

object A Seurat object

tss.positions A GRanges object containing the TSS positions. If NULL, use the genomic

annotations stored in the assay.

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n Number of TSS positions to use. This will select the first \_n\_ TSSs from the set.

If NULL, use all TSSs (slower).

fast Just compute the TSS enrichment score, without storing the base-resolution ma-

trix of integration counts at each site. This reduces the memory required to store

the object but does not allow plotting the accessibility profile at the TSS.

assay Name of assay to use

cells A vector of cells to include. If NULL (default), use all cells in the object

process\_n Number of regions to process at a time if using fast option.

verbose Display messages

region\_extension

Distance extended upstream and downstream from TSS in which to calculate enrichment and background.

#### **Details**

The computed score will be added to the object metadata as "TSS.enrichment".

#### Value

Returns a Seurat object

#### **Examples**

```
## Not run:
fpath <- system.file("extdata", "fragments.tsv.gz", package="Signac")
Fragments(atac_small) <- CreateFragmentObject(
  path = fpath,
  cells = colnames(atac_small),
  tolerance = 0.5
)
TSSEnrichment(object = atac_small)
## End(Not run)</pre>
```

TSSPlot

Plot signal enrichment around TSSs

### Description

Plot the normalized TSS enrichment score at each position relative to the TSS. Requires that TSSEnrichment has already been run on the assay.

# Usage

```
TSSPlot(object, assay = NULL, group.by = NULL, idents = NULL)
```

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#### **Arguments**

object A Seurat object

assay Name of the assay to use. Should have the TSS enrichment information for each

cell already computed by running TSSEnrichment

group.by Set of identities to group cells by idents Set of identities to include in the plot

# Value

Returns a ggplot2 object

UnifyPeaks	Unify genomic ranges

# **Description**

Create a unified set of non-overlapping genomic ranges from multiple Seurat objects containing single-cell chromatin data.

## Usage

```
UnifyPeaks(object.list, mode = "reduce")
```

# Arguments

object.list A list of Seurat objects or ChromatinAssay objects

mode Function to use when combining genomic ranges. Can be "reduce" (default) or

"disjoin". See reduce and disjoin for more information on these functions.

#### Value

Returns a GRanges object

# **Examples**

```
UnifyPeaks(object.list = list(atac_small, atac_small))
```

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UpdatePath	Update the file path for a Fragment object
op aa c c . a c	oparate the fire partings at 1 tagine in object

# **Description**

Change the path to a fragment file store in a Fragment object. Path must be to the same file that was used to create the fragment object. An MD5 hash will be computed using the new path and compared to the hash stored in the Fragment object to verify that the files are the same.

# Usage

```
UpdatePath(object, new.path, verbose = TRUE)
```

# Arguments

object A Fragment object

new.path Path to the fragment file

verbose Display messages

ValidateCells

Validate cells present in fragment file

#### **Description**

Search for a fragment from each cell that should exist in the fragment file. Will iterate through chunks of the fragment file until at least one fragment from each cell barcode requested is found.

# Usage

```
ValidateCells(
  object,
  cells = NULL,
  tolerance = 0.5,
  max.lines = 5e+07,
  verbose = TRUE
)
```

# **Arguments**

object A Fragment object

cells A character vector containing cell barcodes to search for. If NULL, use the cells

stored in the Fragment object.

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tolerance Fraction of input cells that can be unseen before returning TRUE. For example,

tolerance = 0.01 will return TRUE when 99 have observed fragments in the file. This can be useful if there are cells present that have much fewer total counts, and would require extensive searching before a fragment from those

cells are found.

max.lines Maximum number of lines to read in without finding the required number of

cells before returning FALSE. Setting this value avoids having to search the whole file if it becomes clear that the expected cells are not present. Setting this

value to NULL will enable an exhaustive search of the entire file.

verbose Display messages

ValidateFragments Validate Fragment object

# Description

Verify that the cells listed in the object exist in the fragment file and that the fragment file or index have not changed since creating the fragment object.

# Usage

```
ValidateFragments(object, verbose = TRUE, ...)
```

# Arguments

object A Fragment object verbose Display messages

... Additional parameters passed to ValidateCells

Validate Hash Validate hashes for Fragment object

#### **Description**

Validate hashes for Fragment object

# Usage

```
ValidateHash(object, verbose = TRUE)
```

# Arguments

object A Fragment object verbose Display messages 118 VariantPlot

VariantPlot

Plot strand concordance vs. VMR

# Description

Plot the Pearson correlation between allele frequencies on each strand versus the log10 mean-variance ratio for the allele.

#### Usage

```
VariantPlot(
  variants,
  min.cells = 2,
  concordance.threshold = 0.65,
  vmr.threshold = 0.01
)
```

# **Arguments**

variants A dataframe containing variant information. This should be computed using

 ${\tt IdentifyVariants}$ 

min.cells Minimum number of high-confidence cells detected with the variant for the vari-

ant to be displayed.

concordance.threshold

Strand concordance threshold

vmr. threshold Mean-variance ratio threshold

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