Package ‘UMI4Cats’

April 4, 2024

Title  UMI4Cats: Processing, analysis and visualization of UMI-4C chromatin contact data

Version  1.12.0

URL  https://github.com/Pasquali-lab/UMI4Cats

Description  UMI-4C is a technique that allows characterization of 3D chromatin interactions with a bait of interest, taking advantage of a sonication step to produce unique molecular identifiers (UMIs) that help remove duplication bias, thus allowing a better differential comparison of chromatin interactions between conditions. This package allows processing of UMI-4C data, starting from FastQ files provided by the sequencing facility. It provides two statistical methods for detecting differential contacts and includes a visualization function to plot integrated information from a UMI-4C assay.

BugReports  https://github.com/Pasquali-lab/UMI4Cats/issues

License  Artistic-2.0

Encoding  UTF-8

Depends  R (>= 4.0.0), SummarizedExperiment

Imports  magick, cowplot, scales, GenomicRanges, ShortRead, zoo, ggplot2, reshape2, regioneR, IRanges, S4Vectors, magrittr, dplyr, BSgenome, Biostrings, DESeq2, R.utils, Rsamtools, stringr, Rbowtie2, methods, GenomeInfoDb, GenomicAlignments, RColorBrewer, utils, grDevices, stats, org.Hs.eu.db, annotate, TxDb.Hsapiens.UCSC.hg19.knownGene, rlang, GenomicFeatures, BiocFileCache, rappdirs, fda, BiocGenerics

Suggests  knitr, rmarkdown, BiocStyle, BSgenome.Hsapiens.UCSC.hg19, tidyr, testthat

biocViews  QualityControl, Preprocessing, Alignment, Normalization, Visualization, Sequencing, Coverage

VignetteBuilder  knitr

RoxygenNote  7.1.1

git_url  https://git.bioconductor.org/packages/UMI4Cats

git_branch  RELEASE_3_18
R topics documented:

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.getCache  

Get BiocFileCache object

Description

Get BiocFileCache object

Usage

.getCache()

Value

Returns BFC object with the cache for the UMI4Cats package
### .getSummaryBam  
**Summarize BAM file**

**Description**  
Get summary of interesting bam statistics

**Usage**  
```
.getSummaryBam(bam_file, mapped = TRUE, secondary = FALSE)
```

**Arguments**  
- `bam_file`  
  Path for the bam file.
- `mapped`  
  Logical indicating whether to extract mapped reads.
- `secondary`  
  Logical indicating whether to extract secondary aligned reads.

**Value**  
Returns a numeric containing the number of reads in `bam_file` that has the specified mapped and secondary status.

### .singleAlignmentUMI4C  
**Align split fastq file**

**Description**  
Align split fastq file

**Usage**  
```
.singleAlignmentUMI4C(
    split_file,
    align_dir,
    threads = 1,
    bowtie_index,
    pos_viewpoint,
    filter_bp = 1e+07
)
```
Arguments

- **split_file**: Split fastq file to align.
- **align_dir**: Directory where to save aligned files.
- **threads**: Number of threads to use in the analysis. Default=1.
- **bowtie_index**: Path and prefix of the bowtie index to use for the alignment.
- **pos_viewpoint**: GRanges object containing the genomic position of the viewpoint.
- **filter_bp**: Integer indicating the bp upstream and downstream of the viewpoint to select for further analysis. Default=10e6

Value

Creates a BAM file in wk_dir/align named "basename(fastq))_filtered.bam", containing the aligned filtered reads. A data.frame object with the statistics is also returned.

Description

Count UMIs for a given bam file.

Usage

```
.singleCounterUMI4C(
  filtered_bam_R1,
  filtered_bam_R2,
  digested_genome_gr,
  pos_viewpoint,
  res_enz,
  count_dir,
  filter_bp = 1e+07
)
```

Arguments

- **filtered_bam_R1**: R1 bam file.
- **filtered_bam_R2**: R2 bam file.
- **digested_genome_gr**: GRanges object containing the coordinates for the digested genome.
- **pos_viewpoint**: Vector consist of chromosome, start and end position of the viewpoint.
- **res_enz**: Character containing the restriction enzyme sequence.
- **count_dir**: Counter directory.
- **filter_bp**: Integer indicating the bp upstream and downstream of the viewpoint to select for further analysis. Default=10e6
.singlePrepUMI4C

Value

Creates a tab-delimited file in wk_dir/count named "basename(fastq)_counts.tsv", containing the coordinates for the viewpoint fragment, contact fragment and the number of UMIs detected in the ligation.

.singlePrepUMI4C Prepar fastq files at a given barcode.

Description

Prepar fastq files at a given barcode.

Usage

.singlePrepUMI4C(
  fq_R1,
  fq_R2,
  bait_seq,
  bait_pad,
  res_enz,
  prep_dir,
  numb_reads = 1e+09
)

Arguments

fq_R1 Fastq file R1.
fq_R2 Fastq file R2.
bait_seq Character containing the bait primer sequence.
bait_pad Character containing the pad sequence (sequence between the bait primer and the restriction enzyme sequence).
res_enz Character containing the restriction enzyme sequence.
prep_dir Prep directory.
numb_reads Number of lines from the FastQ file to load in each loop. If having memory size problems, change it to a smaller number. Default=1e9.

Value

Creates a compressed FASTQ file in wk_dir/prep named basename(Fastq)).fq.gz, containing the filtered reads with the UMI sequence in the header. A data.frame object with the statistics is also returned.
.singleSplitUMI4C  

**Split fastq files at a given restriction site.**

**Description**

Split fastq files at a given restriction site.

**Usage**

```
.singleSplitUMI4C(
    fastq_file,
    res_enz,
    cut_pos,
    split_dir,
    min_flen = 20,
    numb_reads = 1e+09
)
```

**Arguments**

- `fastq_file`: Fastq file path.
- `res_enz`: Character containing the restriction enzyme sequence.
- `cut_pos`: Numeric indicating the nucleotide position where restriction enzyme cuts (zero-based) (for example, for DpnII is 0).
- `split_dir`: Directory where to save split files.
- `min_flen`: Minimal fragment length to use for selecting the fragments. Default=20
- `numb_reads`: Number of lines from the FastQ file to load in each loop. If having memory size problems, change it to a smaller number. Default=1e9.

**Value**

Creates a compressed FASTQ file in wk_dir/split named basename(fastq)).fq.gz, containing the split reads based on the restriction enzyme used.

---

.smoothMonotone  

**Monotone smoothing of the VST counts**

**Description**

Takes the variance stabilized count values and calculates a symmetric monotone fit for the distance dependency. The signal trend is fitted using the fda package.

**Usage**

```
.smoothMonotone(trafo_counts, alpha = 20, penalty = 0.1, frag_data)
```
**Arguments**

- `trafo_counts`: Variance stabilized count values assay from DDS object.
- `alpha`: Approximate number of fragments desired for every basis function of the B-spline basis. `floor((max(number of fragments)) / alpha)` is passed to `create.bspline.basis` as nbasis argument. 4 is the minimum allowed value. Default: 20.
- `penalty`: Amount of smoothing to be applied to the estimated functional parameter. Default: 0.1.
- `frag_data`: Data frame with all the information on restriction fragments and the interval around the viewpoint.

**Details**

This function computes the smoothing function for the VST values, based on `fda` package, and calculates a symmetric monotone fit counts for the distance dependency

**Value**

A dataframe with monotone smoothed fit counts.

---

**Description**

This function can be used to add specific groupings to UMI4C objects.

**Usage**

```r
addGrouping(
  umi4c,
  grouping = "sampleID",
  scales = 5:150,
  normalized = TRUE,
  sd = 2
)
```

**Arguments**

- `umi4c`: UMI4C object as generated by `makeUMI4C`.
- `grouping`: Name of the column in colData used to merge the samples or replicates. Set to NULL for skipping grouping. Default: "condition".
- `scales`: Numeric vector containing the scales for calculating the domainogram.
- `normalized`: Logical indicating whether UMI-4C profiles should be normalized to the ref_umi4c sample/group. Default: TRUE.
- `sd`: Standard deviation for adaptative trend.
addStepping

Value
 Adds a new UMI4C object into the groupsUMI4C slot with samples grouped according to grouping variable.

Examples

data("ex_ciita_umi4c")

ex_ciita_umi4c <- addGrouping(ex_ciita_umi4c, grouping="condition")

addStepping

Add stepping for plotting genes

Description
 Given a GRanges dataset representing genes, will add an arbitrary value for them to be plotted in the Y axis without overlapping each other.

Usage

addStepping(genesDat, coordinates, mcol.name)

Arguments

genesDat GRanges object containing gene information.
coordinates GRanges object with coordinates you want to plot.
mcol.name Integer containing the column number that contains the gene name.

Value
 Calculates the stepping position to avoid overlap between genes.

alignmentUMI4C

UMI4C alignment

Description
 Align split UMI-4C reads to a reference genome using Bowtie2.

Usage

alignmentUMI4C(
    wk_dir,
    pos_viewpoint,
    bowtie_index,
    threads = 1,
    filter_bp = 1e+07
)
calculateAdaptativeTrend

Adaptative smoothing of normalized trend

Description

Will perform adaptative smoothing will scaling one profile to the reference UMI-4C profile.

Usage

calculateAdaptativeTrend(umi4c, sd = 2, normalized = TRUE)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>umi4c</td>
<td>UMI4C object as generated by <code>makeUMI4C</code>.</td>
</tr>
<tr>
<td>sd</td>
<td>Standard deviation for adaptative trend.</td>
</tr>
<tr>
<td>normalized</td>
<td>Logical indicating whether UMI-4C profiles should be normalized to the <code>ref_umi4c</code> sample/group. Default: TRUE</td>
</tr>
</tbody>
</table>
**calculateDomainogram**

*Create Domainogram*

**Value**
Calculates the adaptative trend considering the minimum number of molecules to use for merging different restriction fragments. It also calculates the geometric mean of the coordinates of the merged restriction fragments.

**Description**
Using as input the raw UMIs, this function creates a domainogram for the supplied scales.

**Usage**
```r
calculateDomainogram(umi4c, scales = 5:150, normalized = TRUE)
```

**Arguments**
- **umi4c** 
  UMI4C object as generated by `makeUMI4C`.
- **scales** 
  Integer vector indicating the number of scales to use for the domainogram creation. Default: 5:150.
- **normalized** 
  Logical whether the resulting domainograms should be normalized or not. Default: TRUE.

**Value**
A matrix where the first column represents the fragment end coordinates (start) and the rest represent the number of UMIs found when using a specific scale.

**callInteractions**
*Call significant interactions*

**Description**
Test a set of `query_regions` for significant interactions with the viewpoint.

**Usage**
```r
callInteractions(
  umi4c,
  design = ~condition,
  query_regions,
  padj_method = "fdr",
  zscore_threshold = 2,
  padj_threshold = 0.1,
  alpha = 20,
  penalty = 0.1
)
```
Arguments

- **umi4c**: UMI4C object as generated by makeUMI4C or the UMI4C constructor.
- **design**: A formula or matrix. The formula expresses how the counts for each fragment end depend on the variables in colData. See DESeqDataSet.
- **query_regions**: GRanges object or data.frame containing the coordinates of the genomic regions you want to use to perform the analysis in specific genomic intervals. Default: NULL.
- **padj_method**: The method to use for adjusting p-values, see p.adjust. Default: fdr.
- **zscore_threshold**: Numeric indicating the z-score threshold to use to define significant differential contacts. Default: 2.
- **padj_threshold**: Numeric indicating the adjusted p-value threshold to use to define significant differential contacts. Default: 0.1.
- **alpha**: Approximate number of fragments desired for every basis function of the B-spline basis. floor(max(number of fragments) / alpha) is passed to create.bspline.basis as nbasis argument. 4 is the minimum allowed value. Default: 20.
- **penalty**: Amount of smoothing to be applied to the estimated functional parameter. Default: 0.1.

Value

**GRangesList** where each element is a UMI4C sample with the queried regions and their adjusted p-values and Z-scores.

Examples

```r
data("ex_ciita_umi4c")
umi <- ex_ciita_umi4c
win_frags <- makeWindowFragments(umi, n_frags=8, sliding=1)

gr <- callInteractions(umi, ~condition, win_frags, padj_threshold = 0.01, zscore_threshold=2)
inter <- getSignInteractions(gr)
```

---

**combineUMI4C**

*Combine UMI4C fragments*

Description

Combine the UMI4C fragments that overlap a given set of query_regions.

Usage

```r
combineUMI4C(umi4c, query_regions)
```
**Arguments**

- **umi4c**
  - UMI4C object as generated by `makeUMI4C` or the UMI4C constructor.

- **query_regions**
  - GRanges object containing the coordinates of the genomic regions for combining restriction fragments.

**Value**

UMI4C object with rowRanges corresponding to query_regions and assay containing the sum of raw UMI counts at each specified query_region.

**Examples**

```r
data("ex_ciita_umi4c")
wins <- makeWindowFragments(ex_ciita_umi4c)
umi_comb <- combineUMI4C(ex_ciita_umi4c, wins)
```

**Description**

Using demultiplexed FastQ files as input, performs all necessary steps to end up with a tsv file summarizing the restriction enzyme fragments and the number of UMIs supporting that specific contact with the viewpoint (bait) of interest.

**Usage**

```r
contactsUMI4C(
  fastq_dir,
  wk_dir,
  file_pattern = NULL,
  bait_seq,
  bait_pad,
  res_enz,
  cut_pos,
  digested_genome,
  bowtie_index,
  threads = 1,
  numb_reads = 1e+09,
  rm_tmp = TRUE,
  min_flen = 20,
  filter_bp = 1e+07,
  ref_gen,
  sel_seqname = NULL
)
```
Arguments

- **fastq_dir**: Path of the directory containing the FastQ files (compressed or uncompressed).
- **wk_dir**: Working directory where to save the outputs generated by the UMI-4c analysis.
- **file_pattern**: Character that can be used to filter the files you want to analyze in the fastq_dir.
- **bait_seq**: Character containing the bait primer sequence.
- **bait_pad**: Character containing the pad sequence (sequence between the bait primer and the restriction enzyme sequence).
- **res_enz**: Character containing the restriction enzyme sequence.
- **cut_pos**: Numeric indicating the nucleotide position where restriction enzyme cuts (zero-based) (for example, for DpnII is 0).
- **digested_genome**: Path for the digested genome file generated using the `digestGenome` function.
- **bowtie_index**: Path and prefix of the bowtie index to use for the alignment.
- **threads**: Number of threads to use in the analysis. Default=1.
- **numb_reads**: Number of lines from the FastQ file to load in each loop. If having memory size problems, change it to a smaller number. Default=1e9.
- **rm_tmp**: Logical indicating whether to remove temporary files (sam and intermediate bams). TRUE or FALSE. Default=TRUE.
- **min_flen**: Minimal fragment length to use for selecting the fragments. Default=20
- **filter_bp**: Integer indicating the bp upstream and downstream of the viewpoint to select for further analysis. Default=10e6
- **ref_gen**: A BSgenome object of the reference genome.
- **sel_seqname**: A character with the chromosome name to focus the search for the viewpoint sequence.

Value

This function is a combination of calls to other functions that perform the necessary steps for processing UMI-4C data.

Examples

```r
if (interactive()) {
  path <- downloadUMI4CexampleData()

  hg19_dpnii <- digestGenome(
    cut_pos = 0,
    res_enz = "GATC",
    name_RE = "DpnII",
    ref_gen = BSgenome.Hsapiens.UCSC.hg19::BSgenome.Hsapiens.UCSC.hg19,
    out_path = file.path(path, "digested_genome")
  )

  raw_dir <- file.path(path, "CIITA", "fastq")
}
```
counterUMI4C

contactsUMI4C(
    fastq_dir = raw_dir,
    wk_dir = file.path(path, "CIITA"),
    bait_seq = "GGACAAGCTCCCTGCAACTCA",
    bait_pad = "GGACTTGCA",
    res_enz = "GATC",
    cut_pos = 0,
    digested_genome = hg19_dpnii,
    bowtie_index = file.path(path, "ref_genome", "ucsc.hg19.chr16"),
    threads = 1,
    numb_reads = 1e9,
    ref_gen = BSgenome.Hsapiens.UCSC.hg19::BSgenome.Hsapiens.UCSC.hg19,
    sel_seqname = "chr16"
)

unlink(path, recursive=TRUE)
}

counterUMI4C  UMI counting

Description

Algorithm for counting and collapsing the number of UMIs supporting a specific ligation.

Usage

counterUMI4C(
    wk_dir,
    pos_viewpoint,
    res_enz,
    digested_genome,
    filter_bp = 1e+07
)

Arguments

wk_dir  Working directory where to save the outputs generated by the UMI-4c analysis.
pos_viewpoint  GRanges object containing the genomic position of the viewpoint.
res_enz  Character containing the restriction enzyme sequence.
digested_genome  Path for the digested genome file generated using the digestGenome function.
filter_bp  Integer indicating the bp upstream and downstream of the viewpoint to select for further analysis. Default=10e6.
createGeneAnnotation

Create gene annotation object

Description

Create gene annotation object

Usage

createGeneAnnotation(
  window,
  TxDb = TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene,
  longest = TRUE
)
createStatsTable

Description
Create a statistical summary of the UMI-4C experiments analyzed with contactsUMI4C.

Usage
createStatsTable(wk_dir)

Arguments
wk_dir Working directory where to save the outputs generated by the UMI-4c analysis.

Value
Returns a data.frame summarizing all the different statistics for each sample analyzed in wk_dir.
darken

**Description**

Darken colors

**Usage**

darken(color, factor = 1.4)

**Arguments**

color Character containing the name or hex value of a color.
factor Numeric representing a factor by which darken the specified color.

**Value**

Darkens the provided color by the provided factor.

**Examples**

darken("blue", factor = 1.4)

---

**dds2UMI4C**

**DDS object to UMI4Cats object.**

**Description**

Transforms an DDS object to a UMI4C object after applying nbinomWaldTestUMI4C.

**Usage**

dds2UMI4C(  
    umi4c,  
    dds,  
    normalized = TRUE,  
    padj_method = "fdr",  
    padj_threshold = 0.05)

)
demultiplexFastq

Arguments

- **umi4c**: UMI4C object as generated by `makeUMI4C` or the UMI4C constructor.
- **dds**: DDS object as generated by `nbinomWaldTestUMI4C` with the DESeq2 Wald Test results.
- **normalized**: Logical indicating if the function should return normalized or raw UMI counts. Default: TRUE.
- **padj_method**: The method to use for adjusting p-values, see `p.adjust`. Default: fdr.
- **padj_threshold**: Numeric indicating the adjusted p-value threshold to use to define significant differential contacts. Default: 0.05.

Value

UMI4C object with the DESeq2 Wald Test results.

---

demultiplexFastq  

Demultiplex FASTQ files using fastq-multx

Description

Demultiplex FASTQ files containing different bait information

Usage

demultiplexFastq(barcodes, fastq, out_path = "raw_fastq", numb_reads = 1e+11)

Arguments

- **barcodes**: Dataframe with "name of sample" and "barcode" for every sample to demultiplex.
- **fastq**: Fastq to demultiplex containing mate 1s. Different pairs should be named as "_R1" or "_R2". Allowed formats: _R1.fastq.gz, _R1.fq.gz, _R1.fastq or _R1.fq.
- **out_path**: Path where to save the demultiplex output. Defaults to a path named `raw_fastq` in your working directory.
- **numb_reads**: Number of lines from the FastQ file to load in each loop. If having memory size problems, change it to a smaller number. Default=10e10.

Value

Paired-end FastQ files demultiplexed in a compressed format. A log file with the statistics is also generated in `out_path` named `barcode_umi4cats_demultiplexFastq_stats.txt`. 
Examples

```r
## Not run:
path <- downloadUMI4CexampleData(use_sample = TRUE)
fastq <- file.path(path, "CIITA", "fastq", "sub_ctrl_hi19_CIITA_R1.fastq.gz")
barcodes <- data.frame(
  sample = c("CIITA"),
  barcode = c("GGACAAGCTCCCTGCAACTCA")
)
demultiplexFastq(
  barcodes = barcodes,
  fastq = fastq,
  out_path = path
)
## End(Not run)
```

---

**dgram**  
*UMI4C class methods*

### Description
This page contains a summary of the different methods used to access the information contained inside the UMI4C-class object. See the details section for more information on the different accessors.

### Usage

```r
dgram(object)
dgram(object) <- value
groupsUMI4C(object, value)
groupsUMI4C(object) <- value
bait(object)
trend(object)
resultsUMI4C(object, format = "GRanges", counts = TRUE, ordered = FALSE)
```

## S4 method for signature 'UMI4C'
dgram(object)

## S4 replacement method for signature 'UMI4C'
dgram(object) <- value
## S4 method for signature 'UMI4C'
groupsUMI4C(object)

## S4 replacement method for signature 'UMI4C'
groupsUMI4C(object) <- value

## S4 method for signature 'UMI4C'
bait(object)

## S4 method for signature 'UMI4C'
trend(object)

## S4 method for signature 'UMI4C'
resultsUMI4C(object, format = "GRanges", counts = FALSE, ordered = FALSE)

### Arguments

- **object**: a UMI4C-class object.
- **value**: Alternative list of dgrams to replace the current slot.
- **format**: Either "GRanges" (default) or "data.frame", indicating the format output of the results.
- **counts**: Logical indicating whether counts for the different region should be provided. Default: FALSE.
- **ordered**: Logical indicating whether to sort output by significance (adjusted p-value). Default: FALSE.

### Value

There are several accessors to easily retrieve information from a UMI4C-class object:

- **dgram**: Returns a named list with the output domainograms for each sample.
- **bait**: Returns a GRanges object with the position of the bait.
- **trend**: Returns a data.frame in long format with the values of the adaptive smoothen trend.
- **resultsUMI4C**: Returns a GRanges or data.frame with the results of the differential analysis.

### See Also

UMI4C, UMI4C-class

### Examples

```r
# Access the different information inside the UMI4C object
data("ex_ciita_umi4c")
ex_ciita_umi4c <- addGrouping(ex_ciita_umi4c, grouping="condition")
dgram(ex_ciita_umi4c)
bait(ex_ciita_umi4c)
```
head(trend(ex_ciita_umi4c))

# Perform differential test
enh <- GRanges(c("chr16:10925006-10928900", "chr16:11102721-11103700"))
umi_dif <- fisherUMI4C(ex_ciita_umi4c, query_regions = enh, filter_low = 20, resize = 5e3)
resultsUMI4C(umi_dif)

differentialNbinomWaldTestUMI4C

Differential UMI4C contacts using DESeq2 Wald Test

Description

Using a UMI4C object, infers the differences between conditions specified in design of the smooth monotone fitted values using a Wald Test from DESeq2 package.

Usage

differentialNbinomWaldTestUMI4C(
  umi4c,
  design = ~condition,
  normalized = TRUE,
  padj_method = "fdr",
  query_regions = NULL,
  padj_threshold = 0.05,
  penalty = 0.1,
  alpha = 20
)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>umi4c</td>
<td>UMI4C object as generated by makeUMI4C or the UMI4C constructor.</td>
</tr>
<tr>
<td>design</td>
<td>A formula or matrix. The formula expresses how the counts for each fragment end depend on the variables in colData. See DESeqDataSet.</td>
</tr>
<tr>
<td>normalized</td>
<td>Logical indicating if the function should return normalized or raw UMI counts. Default: TRUE.</td>
</tr>
<tr>
<td>padj_method</td>
<td>The method to use for adjusting p-values, see p.adjust. Default: fdr.</td>
</tr>
<tr>
<td>query_regions</td>
<td>GRanges object or data.frame containing the coordinates of the genomic regions you want to use to perform the analysis in specific genomic intervals. Default: NULL.</td>
</tr>
<tr>
<td>padj_threshold</td>
<td>Numeric indicating the adjusted p-value threshold to use to define significant differential contacts. Default: 0.05.</td>
</tr>
<tr>
<td>penalty</td>
<td>Amount of smoothing to be applied to the estimated functional parameter. Default: 0.1.</td>
</tr>
<tr>
<td>alpha</td>
<td>Approximate number of fragments desired for every basis function of the B-spline basis. floor((max(number of fragments)) / alpha) is passed to create.bspline.basis as nbasis argument. 4 is the minimum allowed value. Default: 20.</td>
</tr>
</tbody>
</table>
Details

This function fits the signal trend of a variance stabilized count values using a symmetric monotone fit for the distance dependency. Then scales the raw counts across the samples to obtain normalized factors. Finally, it detects differences between conditions applying the DESeq2 Wald Test.

Value

UMI4C object with the DESeq2 Wald Test results.

Examples

```r
## Not run:
files <- list.files(system.file("extdata", "CIITA", "count", package="UMI4Cats"),
    pattern = "*.counts.tsv.gz",
    full.names = TRUE
)
# Create colData including all relevant information
colData <- data.frame(
    sampleID = gsub("_counts.tsv.gz", ",", basename(files)),
    file = files,
    stringsAsFactors = FALSE
)
library(tidyr)
colData <- colData %>%
    separate(sampleID,
        into = c("condition", "replicate", "viewpoint"),
        remove = FALSE
)

# Make UMI-4C object including grouping by condition
umi <- makeUMI4C(
    colData = colData,
    viewpoint_name = "CIITA",
    grouping = NULL,
    bait_expansion = 2e6
)

umi_wald <- differentialNbinomWaldTestUMI4C(umi4c=umi,
    design=~condition,
    alpha = 100)

## End(Not run)
```
Description

Performs an in silico digestion of a given reference genome using a given restriction enzyme sequence.

Usage

digestGenome(
  res_enz,
  cut_pos,
  name_RE,
  ref_gen,
  sel_chr = paste0("chr", c(seq_len(22), "X", "Y")),
  out_path = "digested_genome/"
)

Arguments

res_enz Character containing the restriction enzyme sequence.
cut_pos Numeric indicating the nucleotide position where restriction enzyme cuts (zero-based) (for example, for DpnII is 0).
name_RE Restriction enzyme name.
ref_gen A BSgenome object of the reference genome.
sel_chr Character vector indicating which chromosomes to select for the digestion. Default: chr1-22, chrX, chrY.
out_path Output path where to save the genomic track. The default is a directory named digested_genome/ created in your working directory. The rda objects are saved in folder named by the ref_gene_name_RE in the out_path folder.

Value

Creates a rda file for every chromosome defined in sel_chr.

Examples

library(BSgenome.Hsapiens.UCSC.hg19)
ref_gen <- BSgenome.Hsapiens.UCSC.hg19

hg19_dpnii <- digestGenome(
  res_enz = "GATC",
  cut_pos = 0,
  name_RE = "dpnII",
  sel_chr = "chr16", # Only in chr16 to reduce example running time
  ref_gen = ref_gen,
  out_path = file.path(tempdir(), "digested_genome/")
)
downloadUMI4CexampleData

Description

Downloads the required UMI4Cats example datasets.

Usage

downloadUMI4CexampleData(out_dir = tempdir(), verbose = TRUE, reduced = FALSE)

Arguments

- **out_dir**: Output directory for the datasets, defaults to tempdir().
- **verbose**: Whether to print verbose messages or not. Default: TRUE.
- **reduced**: Whether to use a reduced dataset to make test functions run faster.

Value

It creates the output_dir with the example UMI-4C files used by the vignette and examples. Takes advantage of the BiocFileCache package to make sure that the file has not been previously downloaded by the user.

Examples

```r
if (interactive()) {
  # Using reduced data data to make example faster.
  # Remove reduced=TRUE or set to FALSE to
  # download the full dataset.

  path <- downloadUMI4CexampleData(reduced = TRUE)
}
```

ex_ciita_umi4c

Description

An example UMI4C object showing the contacts with a viewpoint located at the CIITA gene promoter.

Usage

ex_ciita_umi4c
**fisherUMI4C**

**Format**

A UMI4C object from this package.

**Source**

See inst/script/CITA_process_example.R to see the code use for generating the UMI4C object.

---

**fisherUMI4C**

*Differential UMI4C contacts using Fisher's Exact test*

**Description**

Using the UMIs inside query_regions performs Fisher's Exact test to calculate significant differences between contact intensities.

**Usage**

```r
fisherUMI4C(
  umi4c,
  grouping = "condition",
  query_regions,
  resize = NULL,
  window_size = 5000,
  filter_low = 50,
  padj_method = "fdr",
  padj_threshold = 0.05
)
```

**Arguments**

- **umi4c**: UMI4C object as generated by makeUMI4C or the UMI4C constructor.
- **grouping**: Name of the column in colData used to merge the samples or replicates. If none available or want to add new groupings, run addGrouping. Default: "condition".
- **query_regions**: GenomicRanges object or data.frame containing the region coordinates used to perform the differential analysis.
- **resize**: Width in base pairs for resizing the query_regions. Default: no resizing.
- **window_size**: If query_regions are not defined, will bin region in window_size bp and perform the analysis using this windows.
- **filter_low**: Either the minimum median UMIs required to perform Fisher's Exact test or FALSE for performing the test in all windows.
- **padj_method**: Method for adjusting p-values. See p.adjust for the different methods.
- **padj_threshold**: Numeric indicating the adjusted p-value threshold to use to define significant differential contacts.
formatPlotsUMI4C

Details

This function calculates the overlap of fragment ends with either the provided `query_regions` or the binned region using `window_size`. The resulting number of UMIs in each `query_region` will be the sum of UMIs in all overlapping fragment ends. As a default, will filter out those regions whose median UMIs are lower than `filter_low`.

Finally, a contingency table for each `query_regions` or window that passed the `filter_low` filter is created as follows:

<table>
<thead>
<tr>
<th>query_region</th>
<th>region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>n1</td>
</tr>
<tr>
<td>Condition</td>
<td>n2</td>
</tr>
</tbody>
</table>

and the Fisher’s Exact test is performed. Obtained p-values are then converted to adjusted p-values using `padj_method` and the results list is added to the UMI4C object.

Value

Calculates statistical differences between UMI-4C experiments.

Examples

data("ex_ciita_umi4c")
ex_ciita_umi4c <- addGrouping(ex_ciita_umi4c, grouping="condition")

# Perform differential test
enh <- GRanges(c("chr16:10925006-10928900", "chr16:11102721-11103700"))
umi_dif <- fisherUMI4C(ex_ciita_umi4c, query_regions = enh,
                        filter_low = 20, resize = 5e3)
resultsUMI4C(umi_dif)

formatPlotsUMI4C

Description

Format plots for UMI4C

Usage

formatPlotsUMI4C(plot_list, font_size)

Arguments

plot_list List of plots generated by `plotUMI4C`
font_size Base font size to use for the UMI4C plot. Default: 14.
Value

Given a named plot_list and considering the number and type of included plots, formats their axes accordingly to show the final UMI4C plot.

geoMeanCoordinates  Get geometric mean of given coordinates

Description

Get geometric mean of given coordinates

Usage

geoMeanCoordinates(coords, scale, bait_start)

Arguments

cords  Vector of integers representing the coordinates from which to obtain the geometric mean.
scale  Vector of scales indicating how many fragment where merged.
bait_start  Integer indicating the coordinates for the bait start.

Value

Calculates geometric mean of the provided coordinates, taking into account the distance to the viewpoint and how many restriction fragments are being merged.

gColors  Get default colors

Description

Get default colors

Usage

gColors(factors)

Arguments

factors  Name of the factors that will be used for grouping variables.

Value

Depending on the number of factors it creates different color palettes.
getFactors

Get factors for plotting

Description
Get factors for plotting

Usage
getFactors(umi4c, grouping = NULL)

Arguments

- **umi4c**: UMI4C object
- **grouping**: Grouping used for the different samples. If none available or want to add new groupings, run `addGrouping`.

Value
Factor vector where the first element is the reference factor.

getNormalizationMatrix

Get normalization matrix

Description
Will return a normalization matrix.

Usage
generalNormalizationMatrix(
  umi4c,
  norm_bins = 10^(3:6),
  post_smooth_win = 50,
  r_expand = 1.2
)

Arguments

- **umi4c**: UMI4C object as generated by `makeUMI4C`.
- **norm_bins**: Numeric vector with the genomic bins to use for normalization. Default: 1K, 10K, 100K, 1Mb.
- **post_smooth_win**: Numeric indicating the smoothing window to use. Default: 50.
- **r_expand**: Numeric indicating the expansion value for normalization. Default: 1.2.
getViewpointCoordinates

Value
Creates a matrix of normalization factors using as a reference the profile specified in the UMI4C object.

getSignInteractions  Get significant interactions from a GRangesList

Description
Retrieves all significant interactions from the output of callInteractions.

Usage
getSignInteractions(gr_interactions)

Arguments
gr_interactions
GRangesList outputed by callInteractions.

Value
GRanges object with a list of significantly interacting regions.

Examples

data("ex_ciita_um4c")
umi <- ex_ciita_um4c
win_frags <- makeWindowFragments(umi, n_frags=8, sliding=1)

gr <- callInteractions(umi, ~condition, win_frags, padj_threshold = 0.01, zscore_threshold=2)
inter <- getSignInteractions(gr)

getViewpointCoordinates  Get viewpoint coordinates

Description
Finds the viewpoint coordinates for a given reference genome and sequence.
Usage

getViewpointCoordinates(
  bait_seq,
  bait_pad,
  res_enz,
  ref_gen,
  sel_seqname = NULL
)

Arguments

bait_seq Character containing the bait primer sequence.
bait_pad Character containing the pad sequence (sequence between the bait primer and
the restriction enzyme sequence).
res_enz Character containing the restriction enzyme sequence.
ref_gen A BSgenome object of the reference genome.
sel_seqname A character with the chromosome name to focus the search for the viewpoint
sequence.

Value

Creates a GRanges object containing the genomic position of the viewpoint.

Examples

getViewpointCoordinates(
  bait_seq = "GGACAAGCTCCCTGCAACTCA",
  bait_pad = "GGACTTGCA",
  res_enz = "GATC",
  ref_gen = BSgenome.Hsapiens.UCSC.hg19::BSgenome.Hsapiens.UCSC.hg19,
  sel_seqname = "chr16" # Look only in chr16
)

Description

Combines UMI4C samples by adding UMIs from assay(umi4c) to represent the levels in grouping.

Usage

groupSamplesUMI4C(umi4c, grouping = "condition")
**Arguments**

- **umi4c**: UMI4C object as generated by `makeUMI4C`.
- **grouping**: Name of the column in colData used to merge the samples or replicates. Set to NULL for skipping grouping. Default: "condition".

**Value**

A grouped UMI4C object.

---

### Description

Use a set of continuous restriction fragments to generate windows containing a fixed number of fragments (n_frags).

### Usage

```r
makeWindowFragments(input, n_frags = 8, sliding = 1)
```

### Arguments

- **input**: Input object containing the restriction fragments. Should be class UMI4C (rowRanges will be extracted) or class GRanges.
- **n_frags**: Number of fragments to use for generating the windows. This should include restriction fragments with 0 counts (Default: 8).
- **sliding**: Numeric indicating the factor for generating sliding windows. If set to 1 (default) will use fixed windows. If set to > 0 and < 1 will use n_frags * sliding fragments to generate sliding windows.

### Value

A GRanges object containing the windows of merged restriction fragments.

### Examples

```r
data("ex_ciita_umi4c")

# Without sliding windows
win_frags <- makeWindowFragments(ex_ciita_umi4c, n_frags=30, sliding=1)
win_frags

# With sliding windows (n_frags*sliding)
win_frags <- makeWindowFragments(ex_ciita_umi4c, n(frags)=30, sliding=0.5)
win_frags
```
nbinomWaldTestUMI4C

Differential UMI4C contacts using DESeq2 Wald Test

Description
Takes the smooth monotone fit count values and infers the differential UMI4C contacts using DESeq2 Wald Test from DESeq2 package.

Usage
nbinomWaldTestUMI4C(dds, query_regions = NULL)

Arguments
- **dds**: DDS object as generated by smoothMonotoneUMI4C with the smooth monotone fit counts
- **query_regions**: GRanges object or data.frame containing the coordinates of the genomic regions you want to use to perform the analysis in specific genomic intervals. Default: NULL.

Details
This function back-transform fitted values to the scale of raw counts and scale them across the samples to obtain normalized factors using normalizationFactors from DESeq2 package. To detect differences between conditions, the DESeq2 package.

Value
DDS object with the DESeq2 Wald Test results, with results columns accessible with the results function.

plotDifferential

Plot differential contacts

Description
Plot differential contacts

Usage
plotDifferential(umi4c, grouping = NULL, colors = NULL, xlim = NULL)
**plotDomainogram**

**Description**

Plot domainogram

**Usage**

```r
plotDomainogram(
  umi4c, 
  dgram_function = "quotient",
  grouping = NULL, 
  colors = NULL, 
  xlim = NULL)
```

**Arguments**

- `umi4c` **UMI4C object as generated by `makeUMI4C`.**
- `dgram_function` Function used for calculating the fold-change in the domainogram plot, either "difference" or "quotient". Default: "quotient".
- `grouping` Grouping used for the different samples. If none available or want to add new groupings, run `addGrouping`.
- `colors` Named vector of colors to use for plotting for each group.
- `xlim` Limits for the plot x axis (genomic coordinates).

**Examples**

```r
data("ex_ciita_umi4c")
ex_ciita_umi4c <- addGrouping(ex_ciita_umi4c, grouping="condition")

enh <- GRanges(c("chr16:10925006-10928900", "chr16:11102721-11103700"))
umi_dif <- fisherUMI4C(ex_ciita_umi4c, query_regions = enh, 
    filter_low = 20, resize = 5e3)
plotDifferential(umi_dif)
```
plotGenes

Value

Produces the domainogram plot, summarizing the merged number of UMIs at the different scales analyzed (y axis).

Examples

data("ex_ciita_umi4c")
ex_ciita_umi4c <- addGrouping(ex_ciita_umi4c, grouping="condition")
plotDomainogram(ex_ciita_umi4c, grouping = "condition")

plotGenes

Plot genes

Description

Plot genes in a window of interest.

Usage

plotGenes(
  window,
  TxDb = TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene,
  longest = TRUE,
  xlim = NULL,
  font_size = 14
)

Arguments

window  GRanges object with coordinates to use for selecting the genes to plot.
TxDb    TxDb object to use for drawing the genomic annotation.
longest Logical indicating whether to plot only the longest transcripts for each gene in the gene annotation plot.
xlim    Limits for the plot x axis (genomic coordinates).
font_size Base font size to use for the UMI4C plot. Default: 14.

Value

Produces a plot with the genes found in the provided window.

Examples

window <- GRanges("chr16:11348649-11349648")
plotGenes(
  window = window,
  TxDb = TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene
)
plotInteractions

Description
Plot the results of `callInteractions`.

Usage
plotInteractions(gr_interactions, xlim = NULL, significant = TRUE)

Arguments
- `gr_interactions`: GRangesList outputed by `callInteractions`.
- `xlim`: Limits for the plot x axis (genomic coordinates).
- `significant`: Logical indicating whether to plot only significant interactions (default: TRUE).

Value
Produces a ggplot2 plot showing the queried interactions at each analysed sample.

plotInteractionsUMI4C

Description
Plot the results of `callInteractions` together with the gene annotation and the trend.

Usage
plotInteractionsUMI4C(umi4c, gr_interactions, grouping = "condition", significant = TRUE, colors = NULL, xlim = NULL, ylim = NULL, TxDb = TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene, longest = TRUE, rel_heights = c(0.25, 0.5, 0.25), font_size = 14)
**plotTrend**

**Arguments**

- `umi4c`: UMI4C object as generated by `makeUMI4C`.
- `gr_interactions`: GRangesList outputed by `callInteractions`.
- `grouping`: Grouping used for the different samples. If none available or want to add new groupings, run `addGrouping`.
- `significant`: Logical indicating whether to plot only significant interactions (default: TRUE).
- `colors`: Named vector of colors to use for plotting for each group.
- `xlim`: Limits for the plot x axis (genomic coordinates).
- `ylim`: Limits of the trend y axis.
- `TxDb`: TxDb object to use for drawing the genomic annotation.
- `longest`: Logical indicating whether to plot only the longest transcripts for each gene in the gene annotation plot.
- `rel_heights`: Numeric vector of length 3 or 4 (if differential plot) indicating the relative heights of each part of the UMI4C plot.
- `font_size`: Base font size to use for the UMI4C plot. Default: 14.

**Value**

Combined plot with gene annotation, trend and interaction plot.

**Description**

Plot adaptative smoothen trend

**Usage**

```r
plotTrend(umi4c, grouping = NULL, colors = NULL, xlim = NULL, ylim = NULL)
```

**Arguments**

- `umi4c`: UMI4C object as generated by `makeUMI4C`.
- `grouping`: Grouping used for the different samples. If none available or want to add new groupings, run `addGrouping`.
- `colors`: Named vector of colors to use for plotting for each group.
- `xlim`: Limits for the plot x axis (genomic coordinates).
- `ylim`: Limits of the trend y axis.
Value

Produces the adaptative trend plot, showing average UMIs at each position taking into account the minimum number of molecules used to merge restriction fragments.

Examples

data("ex_ciita_umi4c")

plotTrend(ex_ciita_umi4c)

plotUMI4C

Description

Produce a UMI-4C data plot containing the genes in the region, the adaptative smoothen trend and the domainogram.

Usage

plotUMI4C(
  umi4c,
  grouping = "condition",
  dgram_function = "quotient",
  dgram_plot = TRUE,
  colors = NULL,
  xlim = NULL,
  ylim = NULL,
  TxDb = TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene,
  longest = TRUE,
  rel_heights = c(0.25, 0.4, 0.12, 0.23),
  font_size = 14
)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>umi4c</td>
<td>UMI4C object as generated by makeUMI4C.</td>
</tr>
<tr>
<td>grouping</td>
<td>Grouping used for the different samples. If none available or want to add new groupings, run addGrouping.</td>
</tr>
<tr>
<td>dgram_function</td>
<td>Function used for calculating the fold-change in the domainogram plot, either &quot;difference&quot; or &quot;quotient&quot;. Default: &quot;quotient&quot;.</td>
</tr>
<tr>
<td>dgram_plot</td>
<td>Logical indicating whether to plot the domainogram. If the UMI4C object only contains one sample will be automatically set to FALSE. Default: TRUE.</td>
</tr>
<tr>
<td>colors</td>
<td>Named vector of colors to use for plotting for each group.</td>
</tr>
<tr>
<td>xlim</td>
<td>Limits for the plot x axis (genomic coordinates).</td>
</tr>
</tbody>
</table>
prepUMI4C

**Description**

Prepare the FastQ files for the further analysis by selecting reads with bait and adding the respective UMI identifier for each read in its header.

**Usage**

```r
prepUMI4C(
  fastq_dir,
  wk_dir,
  file_pattern = NULL,
  bait_seq,
  bait_pad,
  res_enz,
  numb_reads = 1e+09
)
```

**Arguments**

- `fastq_dir` Path of the directory containing the FastQ files (compressed or uncompressed).
- `wk_dir` Working directory where to save the outputs generated by the UMI-4c analysis.
- `file_pattern` Character that can be used to filter the files you want to analyze in the `fastq_dir`.

**Value**

Produces a summary plot with all the information contained in the UMI4C object.

**Examples**

```r
data("ex_ciita_umi4c")
ex_ciita_umi4c <- addGrouping(ex_ciita_umi4c, grouping="condition")

plotUMI4C(ex_ciita_umi4c,
  dgram_plot = FALSE
)
```
smoothMonotoneUMI4C

Description

Takes the variance stabilized count values and calculates a symmetric monotone fit for the distance dependency. The signal trend is fitted using the *fda* package. The position information about the viewpoint have to be stored in the metadata as metadata(dds)['bait'].

Usage

smoothMonotoneUMI4C(dds, alpha = 20, penalty = 0.1)
splitUMI4C

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>dds</td>
<td>DDS object as generated by vstUMI4C with the variance stabilized count values.</td>
</tr>
<tr>
<td>alpha</td>
<td>Approximate number of fragments desired for every basis function of the B-spline basis. floor((max(number of fragments)) / alpha) is passed to create.bspline.basis as nbasis argument. 4 is the minimum allowed value. Default: 20.</td>
</tr>
<tr>
<td>penalty</td>
<td>Amount of smoothing to be applied to the estimated functional parameter. Default: 0.1.</td>
</tr>
</tbody>
</table>

Details

This function computes the smoothing function for the VST values, based on fda package, and calculates a symmetric monotone fit counts for the distance dependency.

Value

DDS object with monotone smoothed fit counts.

splitUMI4C  Split UMI4C reads

Description

Split the prepared reads using the restriction enzyme information.

Usage

splitUMI4C(wk_dir, res_enz, cut_pos, numb_reads = 1e+09, min_flen = 20)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>wk_dir</td>
<td>Working directory where to save the outputs generated by the UMI-4c analysis.</td>
</tr>
<tr>
<td>res_enz</td>
<td>Character containing the restriction enzyme sequence.</td>
</tr>
<tr>
<td>cut_pos</td>
<td>Numeric indicating the nucleotide position where restriction enzyme cuts (zero-based) (for example, for DpnII is 0).</td>
</tr>
<tr>
<td>numb_reads</td>
<td>Number of lines from the FastQ file to load in each loop. If having memory size problems, change it to a smaller number. Default=1e9.</td>
</tr>
<tr>
<td>min_flen</td>
<td>Minimal fragment length to use for selecting the fragments. Default=20</td>
</tr>
</tbody>
</table>

Value

Creates a compressed FASTQ file in wk_dir/split named basename(fastq)).fq.gz, containing the split reads based on the restriction enzyme used.
Examples

```r
if (interactive()) {
  path <- downloadUMI4CexampleData(reduced = TRUE)

  splitUMI4C(
    wk_dir = file.path(path, "CIITA"),
    res_enz = "GATC",
    cut_pos = 0
  )
}
```

---

**statsUMI4C**  
*Statistics UMI4C*

**Description**

Creates a stats summary file and generates a summary plot describing statistics for processed UMI-4C samples.

**Usage**

```r
statsUMI4C(wk_dir)
```

**Arguments**

- `wk_dir`  
  Working directory where to save the outputs generated by the UMI-4C analysis.

**Value**

Returns a plot summarizing the main statistics of the processed UMI-4C experiments found in `wk_dir`. Also, writes a file named `stats_summary.txt` in `wk_dir/logs` that summarizes all the values represented in the plot.

**Examples**

```r
statsUMI4C(wk_dir = system.file("extdata", "CIITA", package = "UMI4Cats")
))
stats <- read.delim(system.file("extdata", "CIITA", "logs", "stats_summary.txt", package = "UMI4Cats")
))
head(stats)
```
theme

Description
Theme

Usage
theme(...)

Arguments
... Additional arguments to pass to the theme call from ggplot2.

Value
ggplot2 theme.

Examples
library(ggplot2)

ggplot(
  iris,
  aes(Sepal.Length, Sepal.Width)
) +
  geom_point() +
  theme()

themeXblank

Description
Theme X blank

Usage
themeXblank(...)

Arguments
... Additional arguments to pass to the theme call from ggplot2.

Value
ggplot2 theme with a blank X axis.
Examples

```r
library(ggplot2)

ggplot(
  iris,
  aes(Sepal.Length, Sepal.Width)
) +
  geom_point() +
  themeXYblank()
```

### Description

Theme Y blank

### Usage

```
themeXYblank(...)
```

### Arguments

... Additional arguments to pass to the theme call from `ggplot2`.

### Value

`ggplot2` theme with a blank X and Y axis.

### Examples

```r
library(ggplot2)

ggplot(
  iris,
  aes(Sepal.Length, Sepal.Width)
) +
  geom_point() +
  themeXYblank()
```
**themeYblank**

*Theme Y blank*

**Description**
Theme Y blank

**Usage**
themeYblank(...)

**Arguments**
... Additional arguments to pass to the theme call from ggplot2.

**Value**

ggplot2 theme with a blank Y axis.

**Examples**
library(ggplot2)

```r
ggplot(
  iris,
  aes(Sepal.Length, Sepal.Width)
) +
  geom_point() +
  themeYblank()
```

---

**UMI4C**

*UMI4C-class*

**Description**
The **UMI4C** constructor is the function `makeUMI4C`. By using the arguments listed below, performs the necessary steps to analyze UMI-4C data and summarize it in an object of class **UMI4C**.

**Usage**
makeUMI4C(
  colData,
  viewpoint_name = "Unknown",
  grouping = "condition",
  normalized = TRUE,
  ref_um4c = NULL,
  bait_exclusion = 3000,
)
bait_expansion = 1e+06,
scales = 5:150,
min_win_factor = 0.02,
sd = 2
)

Arguments

colData Data.frame containing the information for constructing the UMI4C experiment object. Needs to contain the following columns:
- sampleID. Unique identifier for the sample.
- condition. Condition for performing differential analysis. Can be control and treatment, two different cell types, etc.
- replicate. Number for identifying replicates.
- file. File as outputed by umi4CatsContacts function.

viewpoint_name Character indicating the name for the used viewpoint.

grouping Name of the column in colData used to merge the samples or replicates. Set to NULL for skipping grouping. Default: "condition".

normalized Logical indicating whether UMI-4C profiles should be normalized to the ref_umi4c sample/group. Default: TRUE

ref_umi4c Name of the sample or group to use as reference for normalization. By default is NULL, which means it will use the sample with less UMIs in the analyzed region. It should be a named vector, where the name corresponds to the grouping column from colData and the value represents the level to use as reference.

bait_exclusion Region around the bait (in bp) to be excluded from the analysis. Default: 3000bp.

bait_expansion Number of bp upstream and downstream of the bait to use for the analysis (region centered in bait). Default: 1Mb.

scales Numeric vector containing the scales for calculating the domainogram.

min_win_factor Proportion of UMIs that need to be found in a specific window for adaptative trend calculation

sd Standard deviation for adaptative trend.

Value

It returns an object of the class UMI4C.

Slots

colData Data.frame containing the information for constructing the UMI4C experiment object.
Needs to contain the following columns:
- sampleID: Unique identifier for the sample.
- condition: Condition for performing differential analysis. Can be control and treatment, two different cell types, etc.
- replicate: Number or ID for identifying different replicates.
- file: Path to the files outputed by contactsUMI4C.
rowRanges  GRanges object with the coordinates for the restriction fragment ends, their IDs and other additional annotation columns.

metadata  List containing the following elements:
  1. bait: GRanges object representing the position of the bait used for the analysis.
  2. scales: Numeric vector containing the scales used for calculating the domainogram.
  3. min_win_factor: Factor for calculating the minimum molecules required in a window for not merging it with the next one when calculating the adaptive smoothing trend.
  4. grouping: Columns in colData used for the different sample groupings, accessible through groupsUMI4C.
  5. normalized: Logical indicating whether samples/groups are normalized or not.
  6. region: GRanges with the coordinates of the genomic window used for analyzing UMI4C data.
  7. ref_umi4c: Name of the sample or group used as reference for normalization.

assays  Matrix where each row represents a restriction fragment site and columns represent each sample or group defined in grouping. After running the makeUMI4C function, it will contain the following data:
  1. umis: Raw number of UMIs detected by contactsUMI4C.
  2. norm_mat: Normalization factors for each sample/group and fragment end.
  3. trend: Adaptive smoothing trend of UMIs.
  4. geo_coords: Geometric coordinates obtained when performing the adaptive smoothing.
  5. scale: Scale selected for the adaptive smoothing.
  6. sd: Standard deviation for the adaptive smoothing trend.

dgram  List containing the domainograms for each sample. A domainogram is matrix where columns are different scales selected for merging UMI counts and rows are the restriction fragments.

groupsUMI4C  List of UMI4C objects with the specific groupings.

results  List containing the results for the differential analysis ran using fisherUMI4C.

Note
The UMI4C class extends the SummarizedExperiment class.

See Also
UMI4C-methods

Examples

# Load sample processed file paths
files <- list.files(system.file("extdata", "CIITA", "count", 
  package = "UMI4Cats"
),
pattern = "*_counts.tsv",
full.names = TRUE
)
# Create colData including all relevant information
colData <- data.frame(
    sampleID = gsub("_counts.tsv.gz", ",", basename(files)),
    file = files,
    stringsAsFactors = FALSE
)

library(tidyr)
colData <- colData %>%
    separate(sampleID,
        into = c("condition", "replicate", "viewpoint"),
        remove = FALSE
)

# Load UMI-4C data and generate UMI4C object
umi <- makeUMI4C(
    colData = colData,
    viewpoint_name = "CIITA",
    grouping = "condition"
)

---

UMI4C2dds  

**UMI4Cats object to DDS object.**

**Description**

Transforms an UMI4C object to a DDS object

**Usage**

UMI4C2dds(umi4c, design = ~condition)

**Arguments**

- umi4c: UMI4C object as generated by makeUMI4C or the UMI4C constructor.
- design: A formula or matrix. The formula expresses how the counts for each fragment end depend on the variables in colData. See DESeqDataSet.

**Value**

DDS object.
UMI4Cats: A package for analyzing UMI-4C chromatin contact data

Description

The UMI4Cats package provides functions for the pre-processing, analysis and visualization of UMI-4C chromatin contact data.

File preparation

There are two different functions that can be used to prepare the files for analyzing them with UMI4Cats:

1. `demultiplexFastq`. Demultiplex reads belonging to different viewpoints from a paired-end FastQ file.
2. `digestGenome`. Digest the reference genome of choice using a given restriction sequence.

Processing

The pre-processing functions are wrapped in the `contactsUMI4C` main function. This function will sequentially run the following steps:

1. `prepUMI4C`. Filter specific and high quality reads.
2. `splitUMI4C`. Split reads by the restriction sequence.
3. `alignmentUMI4C`. Align reads to the reference genome.
4. `counterUMI4C`. Apply UMI counting algorithm to quantify the interactions with the viewpoint.

The statistics from the samples analyzed with the `contactsUMI4C` function can be extracted and visualized with the function `statsUMI4C`.

Analysis

The analysis of UMI-4C data is wrapped in the construction of an object of UMI4C class by the creator function `makeUMI4C`. This function will group your samples according to the variable you provided in the `grouping` argument (default: "condition") and then normalize it to `ref_umi4c`.

Significant interactions with the viewpoint can be called when several replicates are available, using the `callInteractions` function. A set of `query_regions`, such as enhancers or open chromatin regions needs to be provided. When no candidate regions are available, one can use the function `makeWindowFragments` to join a fixed number of restriction fragments into windows. The results of this analysis can be visualized using `plotInteractionsUMI4C` and the list of significant interactions can be retrieved using `getSignInteractions`.

The differential analysis can be performed with `fisherUMI4C` or `waldUMI4C` functions and can be focused in a set of regions of interest by providing the `query_regions` argument. Both will return a UMI4C object containing the results of the differential test. You can access these results with the method `resultsUMI4C`. 
Visualization

An integrative plot showing the results stored inside the UMI4C object can be generated with the function `plotUMI4C`.

---

vstUMI4C  
Variance stabilizing transformation

Description

Using a DDS object performs a variance stabilizing transformation from DESeq2 package to the UMI4C counts

Usage

vstUMI4C(dds)

Arguments

dds  DDS object generated by UMI4C2dds

Details

This function estimate the size factors and dispersions of the counts base on DESeq for infering the VST distribution and transform raw UMI4C counts.

Value

DDS object with variance stabilizing transformation counts

---

waldUMI4C  
DESeq2 Wald test for differential contacts

Description

Using a UMI4C object, infers the differences between conditions specified in design using a Wald Test from DESeq2 package.

Usage

waldUMI4C(
  umi4c,
  query_regions = NULL,
  subset = "sum",
  design = ~condition,
  normalized = TRUE,
  padj_method = "fdr",
  padj_threshold = 0.05
)
Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>umi4c</td>
<td>UMI4C object as generated by makeUMI4C or the UMI4C constructor.</td>
</tr>
<tr>
<td>query_regions</td>
<td>GRanges object containing the coordinates of the genomic regions you want to use to perform the analysis in specific genomic intervals. Default: NULL.</td>
</tr>
<tr>
<td>subset</td>
<td>If query_regions are provided, how to subset the UMI4C object: &quot;sum&quot; for summing raw UMIs in fragments overlapping query_regions (default) or &quot;overlap&quot; for selecting overlapping fragments.</td>
</tr>
<tr>
<td>design</td>
<td>A formula or matrix. The formula expresses how the counts for each fragment end depend on the variables in colData. See DESeqDataSet.</td>
</tr>
<tr>
<td>normalized</td>
<td>Logical indicating if the function should return normalized or raw UMI counts. Default: TRUE.</td>
</tr>
<tr>
<td>padj_method</td>
<td>The method to use for adjusting p-values, see p.adjust. Default: fdr.</td>
</tr>
<tr>
<td>padj_threshold</td>
<td>Numeric indicating the adjusted p-value threshold to use to define significant differential contacts. Default: 0.05.</td>
</tr>
</tbody>
</table>

Value

UMI4C object with the DESeq2 Wald Test results, which can be accessed using resultsUMI4C.

Examples

```r
data("ex_ciita_umi4c")

umi_dif <- waldUMI4C(ex_ciita_umi4c)
```

Description

Calculates the z-score and then they are converted into one-sided P-values and adjusted for multiple testing using the method of Benjamini and Hochberg.

Usage

```r
zscoreUMI4C(  
  dds,  
  padj_method = "fdr",  
  zscore_threshold = 2,  
  padj_threshold = 0.1  
)`
Arguments

- **dds**: DDS object as generated by `smoothMonotoneUMI4C` with the smooth monotone fit counts.
- **padj_method**: The method to use for adjusting p-values, see `p.adjust`. Default: `fdr`.
- **zscore_threshold**: Numeric indicating the z-score threshold to use to define significant differential contacts. Default: 2.
- **padj_threshold**: Numeric indicating the adjusted p-value threshold to use to define significant differential contacts. Default: 0.1.

Details

This function calculates the z-score for each fragment over all samples from the residuals of the symmetric monotone fit and the median absolute deviation (mad). Z-scores are then converted into one-sided P-values using the standard Normal cumulative distribution function, and these are adjusted for multiple testing using the method of Benjamini and Hochberg.

Value

DDS object with zscore, pvalue and padjusted assays

%>% Pipe operator

Description

See magrittr::%>% for details.

Usage

lhs %>% rhs

Value

Use pipe (magrittr::%>) inside the package.

Examples

```r
mtcars %>%
dplyr::select(cyl)
```
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