

# Package ‘UMI4Cats’

April 14, 2025

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

**Version** 1.17.0

**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>

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**Encoding** UTF-8

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**Author** Mireia Ramos-Rodriguez [aut, cre] (ORCID:  
<https://orcid.org/0000-0001-8083-2445>),  
 Marc Subirana-Granes [aut],  
 Lorenzo Pasquali [aut]

**Maintainer** Mireia Ramos-Rodriguez <mireiarr9@gmail.com>

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|                        |                                 |
|------------------------|---------------------------------|
| <code>.getCache</code> | <i>Get BiocFileCache object</i> |
|------------------------|---------------------------------|

---

## Description

Get BiocFileCache object

## Usage

```
.getCache()
```

## Value

Returns BFC object with the cache for the UMI4Cats package

---

|                             |                           |
|-----------------------------|---------------------------|
| <code>.getSummaryBam</code> | <i>Summarize BAM file</i> |
|-----------------------------|---------------------------|

---

### Description

Get summary of interesting bam statistics

### Usage

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

### Arguments

|                        |                                                                |
|------------------------|----------------------------------------------------------------|
| <code>bam_file</code>  | Path for the bam file.                                         |
| <code>mapped</code>    | Logical indicating whether to extract mapped reads.            |
| <code>secondary</code> | 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.

---

|                                    |                               |
|------------------------------------|-------------------------------|
| <code>.singleAlignmentUMI4C</code> | <i>Align split fastq file</i> |
|------------------------------------|-------------------------------|

---

### Description

Align split fastq file

### Usage

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

**Arguments**

|                            |                                                                                                                 |
|----------------------------|-----------------------------------------------------------------------------------------------------------------|
| <code>split_file</code>    | Split fastq file to align.                                                                                      |
| <code>align_dir</code>     | Directory where to save aligned files.                                                                          |
| <code>threads</code>       | Number of threads to use in the analysis. Default=1.                                                            |
| <code>bowtie_index</code>  | Path and prefix of the bowtie index to use for the alignment.                                                   |
| <code>pos_viewpoint</code> | GRanges object containing the genomic position of the viewpoint.                                                |
| <code>filter_bp</code>     | 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.

---

|                                  |                                         |
|----------------------------------|-----------------------------------------|
| <code>.singleCounterUMI4C</code> | <i>Count UMIs for a given bam file.</i> |
|----------------------------------|-----------------------------------------|

---

**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**

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

**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.

---

|                               |                                               |
|-------------------------------|-----------------------------------------------|
| <code>.singlePrepUMI4C</code> | <i>Prepar fastq files at a given barcode.</i> |
|-------------------------------|-----------------------------------------------|

---

**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**

|                         |                                                                                                                                       |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| <code>fq_R1</code>      | Fastq file R1.                                                                                                                        |
| <code>fq_R2</code>      | Fastq file R2.                                                                                                                        |
| <code>bait_seq</code>   | Character containing the bait primer sequence.                                                                                        |
| <code>bait_pad</code>   | Character containing the pad sequence (sequence between the bait primer and the restriction enzyme sequence).                         |
| <code>res_enz</code>    | Character containing the restriction enzyme sequence.                                                                                 |
| <code>prep_dir</code>   | Prep directory.                                                                                                                       |
| <code>numb_reads</code> | 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.

---

|                                |                                                       |
|--------------------------------|-------------------------------------------------------|
| <code>.singleSplitUMI4C</code> | <i>Split fastq files at a given restriction site.</i> |
|--------------------------------|-------------------------------------------------------|

---

**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**

|                         |                                                                                                                                       |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| <code>fastq_file</code> | Fastq file path.                                                                                                                      |
| <code>res_enz</code>    | Character containing the restriction enzyme sequence.                                                                                 |
| <code>cut_pos</code>    | Numeric indicating the nucleotide position where restriction enzyme cuts (zero-based) (for example, for DpnII is 0).                  |
| <code>split_dir</code>  | Directory where to save split files.                                                                                                  |
| <code>min_flen</code>   | Minimal fragment length to use for selecting the fragments. Default=20                                                                |
| <code>numb_reads</code> | 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.

---

|                              |                                             |
|------------------------------|---------------------------------------------|
| <code>.smoothMonotone</code> | <i>Monotone smoothing of the VST counts</i> |
|------------------------------|---------------------------------------------|

---

**Description**

Takes the variance stabilized count values and calculates a symmetric monotone fit for the distance dependency. The signal trend is fitted using the `fd` 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. $\text{floor}((\max(\text{number of fragments})) / \alpha)$ is passed to <code>create.bspline.basis</code> as <code>nbasis</code> 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.

---

|             |                                       |
|-------------|---------------------------------------|
| addGrouping | <i>Add grouping of UMI-4C samples</i> |
|-------------|---------------------------------------|

---

**Description**

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

**Usage**

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

**Arguments**

|            |                                                                                                                                                           |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| umi4c      | <a href="#">UMI4C</a> object as generated by <a href="#">makeUMI4C</a> .                                                                                  |
| grouping   | Name of the column in <code>colData</code> used to merge the samples or replicates. Set to <code>NULL</code> 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 <code>ref_umi4c</code> sample/group. Default: <code>TRUE</code>                    |
| sd         | Stantard deviation for adaptative trend.                                                                                                                  |



**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 | <i>Add stepping for plotting genes</i> |
|-------------|----------------------------------------|

---

**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 | <i>UMI4C alignment</i> |
|----------------|------------------------|

---

**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
)
```

**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. It can be generated by getViewpointCoordinates 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.                                                                      |
| 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. The alignment log is also generated in wk\_dir/logs named "umi4c\_alignment\_stats.txt".

**Examples**

```
if (interactive()){
  path <- downloadUMI4CexampleData(reduced = TRUE)
  alignmentUMI4C(
    wk_dir = file.path(path, "CIITA"),
    pos_viewpoint = GenomicRanges::GRanges("chr16:10972515-10972548"),
    bowtie_index = file.path(path, "ref_genome", "ucsc.hg19.chr16")
  )
}
```

---

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**

|            |                                                                                                              |
|------------|--------------------------------------------------------------------------------------------------------------|
| umi4c      | <a href="#">UMI4C</a> object as generated by <a href="#">makeUMI4C</a> .                                     |
| sd         | Stantard deviation for adaptative trend.                                                                     |
| normalized | Logical indicating whether UMI-4C profiles should be normalized to the ref_umi4c sample/group. Default: TRUE |

**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.

---

|                      |                           |
|----------------------|---------------------------|
| calculateDomainogram | <i>Create Domainogram</i> |
|----------------------|---------------------------|

---

**Description**

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

**Usage**

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

**Arguments**

|            |                                                                                                     |
|------------|-----------------------------------------------------------------------------------------------------|
| umi4c      | UMI4C object as generated by <a href="#">makeUMI4C</a> .                                            |
| scales     | Integer vector indicating the number of scales to use for the domainogram creation. Default: 5:150. |
| normalized | Logical whether the 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 | <i>Call significant interactions</i> |
|------------------|--------------------------------------|

---

**Description**

Test a set of query\_regions for significant interactions with the viewpoint.

**Usage**

```
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 <a href="#">DESeqDataSet</a> .                                                                                                                      |
| 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 <a href="#">p.adjust</a> . 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. <code>floor((max(number of fragments)) / alpha)</code> is passed to <code>create.bspline.basis</code> as <code>nbasis</code> 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**

```
data("ex_ciita_umi4c")
umi <- ex_ciita_umi4c
win_fragments <- makeWindowFragments(umi, n_fragments=8, sliding=1)

gr <- callInteractions(umi, ~condition, win_fragments, 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**

```
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**

```
data("ex_ciita_umi4c")

wins <- makeWindowFragments(ex_ciita_umi4c)
umi_comb <- combineUMI4C(ex_ciita_umi4c, wins)
```

---

|               |                                  |
|---------------|----------------------------------|
| contactsUMI4C | <i>UMI4C Contacts Processing</i> |
|---------------|----------------------------------|

---

**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**

```
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 <a href="#">digestGenome</a> 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**

```

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")

```

```

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 <a href="#">digestGenome</a> function.                     |
| filter_bp       | Integer indicating the bp upstream and downstream of the viewpoint to select for further analysis. Default=10e6. |

**Details**

For collapsing different molecules into the same UMI, takes into account the ligation position and the number of UMI sequence mismatches.

**Value**

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

**Examples**

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

  hg19_dpnii <- digestGenome(
    cut_pos = 0,
    res_enz = "GATC",
    name_RE = "DpnII",
    sel_chr = "chr16", # digest only chr16 to make example faster
    ref_gen = BSgenome.Hsapiens.UCSC.hg19::BSgenome.Hsapiens.UCSC.hg19,
    out_path = file.path(path, "digested_genome")
  )

  viewpoint <- GenomicRanges::GRanges("chr16:10972515-10972548")

  counterUMI4C(
    wk_dir = file.path(path, "CIITA"),
    pos_viewpoint = viewpoint,
    res_enz = "GATC",
    digested_genome = hg19_dpnii
  )
}
```

---

`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
)
```



**Arguments**

|         |                                                                                                            |
|---------|------------------------------------------------------------------------------------------------------------|
| window  | <a href="#">GRanges</a> 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. |

**Value**

GRanges object with the gene annotation in the window.

**Examples**

```
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
window <- GRanges("chr16:11298262-11400036")
gene_anno <- createGeneAnnotation(
  window = window,
  TxDb = TxDb.Hsapiens.UCSC.hg19.knownGene
)
```

---

|                  |                           |
|------------------|---------------------------|
| createStatsTable | <i>Create stats table</i> |
|------------------|---------------------------|

---

**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 | <i>Darken colors</i> |
|--------|----------------------|

---

### 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 | <i>DDS object to UMI4Cats object.</i> |
|-----------|---------------------------------------|

---

### 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
)
```

**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 <a href="#">p.adjust</a> . 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 | <i>Demultiplex FASTQ files using fastq-multx</i> |
|------------------|--------------------------------------------------|

---

**Description**

Demultiplex FASTQ files containng 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

```
## Not run:
path <- downloadUMI4CexampleData(use_sample = TRUE)
fastq <- file.path(path, "CIITA", "fastq", "sub_ctrl_hi19_CIIITA_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

```
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

```
# 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

|                |                                                                                                                                                                                                                                                                             |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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 <a href="#">DESeqDataSet</a> .                                                                                                                      |
| 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 <a href="#">p.adjust</a> . Default: fdr.                                                                                                                                                                                      |
| 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_threshold | Numeric indicating the adjusted p-value threshold to use to define significant differential contacts. Default: 0.05.                                                                                                                                                        |
| penalty        | Amount of smoothing to be applied to the estimated functional parameter. Default: 0.1.                                                                                                                                                                                      |
| alpha          | Approximate number of fragments desired for every basis function of the B-spline basis. <code>floor((max(number of fragments)) / alpha)</code> is passed to <code>create.bspline.basis</code> as <code>nbasis</code> argument. 4 is the minimum allowed value. Default: 20. |

## 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

```
## 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

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

*Download UMI4Cats example datasets*


---

### 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

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

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

---

ex\_ciita\_umi4c

*Contacs with CIITA promoter*


---

### Description

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

### Usage

```
ex_ciita_umi4c
```

**Format**

A UMI4C object from this package.

**Source**

See `inst/script/CIITA_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**

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

**Arguments**

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

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\_reegions or window that passed the filter\_low filter is created as follows:

|                  | <i>query_region</i> | <i>region</i> |
|------------------|---------------------|---------------|
| <i>Reference</i> | n1                  | N1-n1         |
| <i>Condition</i> | n2                  | N2-n2         |

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 | <i>Format plots for UMI4C</i> |
|------------------|-------------------------------|

---

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.

---

|                                 |                                                |
|---------------------------------|------------------------------------------------|
| <code>geoMeanCoordinates</code> | <i>Get geometric mean of given coordinates</i> |
|---------------------------------|------------------------------------------------|

---

**Description**

Get geometric mean of given coordinates

**Usage**

```
geoMeanCoordinates(coords, scale, bait_start)
```

**Arguments**

|                         |                                                                                          |
|-------------------------|------------------------------------------------------------------------------------------|
| <code>coords</code>     | Vector of integers representing the coordinates from which to obtain the geometric mean. |
| <code>scale</code>      | Vector of scales indicating how many fragment where merged.                              |
| <code>bait_start</code> | 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.

---

|                        |                           |
|------------------------|---------------------------|
| <code>getColors</code> | <i>Get default colors</i> |
|------------------------|---------------------------|

---

**Description**

Get default colors

**Usage**

```
getColors(factors)
```

**Arguments**

|                      |                                                               |
|----------------------|---------------------------------------------------------------|
| <code>factors</code> | Name of the factors that will be used for grouping variables. |
|----------------------|---------------------------------------------------------------|

**Value**

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

---

|            |                                 |
|------------|---------------------------------|
| getFactors | <i>Get factors fro plotting</i> |
|------------|---------------------------------|

---

**Description**

Get factors fro 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 <a href="#">addGrouping</a> . |

**Value**

Factor vector where the first element is the reference factor.

---

|                        |                                 |
|------------------------|---------------------------------|
| getNormalizationMatrix | <i>Get normalization matrix</i> |
|------------------------|---------------------------------|

---

**Description**

Will return a normalization matrix.

**Usage**

```
getNormalizationMatrix(  
  umi4c,  
  norm_bins = 10^(3:6),  
  post_smooth_win = 50,  
  r_expand = 1.2  
)
```

**Arguments**

|                 |                                                                                             |
|-----------------|---------------------------------------------------------------------------------------------|
| umi4c           | <a href="#">UMI4C</a> object as generated by <a href="#">makeUMI4C</a> .                    |
| 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 indicanting the expansion value for normalization. Default: 1.2.                    |

**Value**

Creates a matrix of normalization factors using as a reference the profile specified in the [UMI4C](#) object.

---

|                     |                                                        |
|---------------------|--------------------------------------------------------|
| getSignInteractions | <i>Get significant interactions from a GRangesList</i> |
|---------------------|--------------------------------------------------------|

---

**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_umi4c")
umi <- ex_ciita_umi4c
win_frgs <- makeWindowFragments(umi, n_frgs=8, sliding=1)

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

---

|                         |                                  |
|-------------------------|----------------------------------|
| getViewpointCoordinates | <i>Get viewpoint coordinates</i> |
|-------------------------|----------------------------------|

---

**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  
)
```

---

|                   |                            |
|-------------------|----------------------------|
| groupSamplesUMI4C | <i>Group UMI4C samples</i> |
|-------------------|----------------------------|

---

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 <code>makeUMI4C</code> .                                                                           |
| 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.

---

|                     |                                                   |
|---------------------|---------------------------------------------------|
| makeWindowFragments | <i>Make windows merging restriction fragments</i> |
|---------------------|---------------------------------------------------|

---

**Description**

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

**Usage**

```
makeWindowFragments(input, n_fragments = 8, sliding = 1)
```

**Arguments**

|             |                                                                                                                                                                                                         |
|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| input       | Input object containing the restriction fragments. Should be class UMI4C (rowRanges will be extracted) or class GRanges.                                                                                |
| n_fragments | 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_fragments * sliding fragments to generate sliding windows. |

**Value**

A GRanges object containing the windows of merged restriction fragments.

**Examples**

```
data("ex_ciita_umi4c")

# Without sliding windows
win_fragments <- makeWindowFragments(ex_ciita_umi4c, n_fragments=30, sliding=1)
win_fragments

# With sliding windows (n_fragments*sliding)
win_fragments <- makeWindowFragments(ex_ciita_umi4c, n_fragments=30, sliding=0.5)
win_fragments
```



---

|                     |                                                           |
|---------------------|-----------------------------------------------------------|
| nbinomWaldTestUMI4C | <i>Differential UMI4C contacts using DESeq2 Wald Test</i> |
|---------------------|-----------------------------------------------------------|

---

### 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

### Value

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

---

|                  |                                   |
|------------------|-----------------------------------|
| plotDifferential | <i>Plot differential contacts</i> |
|------------------|-----------------------------------|

---

### Description

Plot differential contacts

### Usage

```
plotDifferential(umi4c, grouping = NULL, colors = NULL, xlim = NULL)
```

**Arguments**

|          |                                                                                                                             |
|----------|-----------------------------------------------------------------------------------------------------------------------------|
| umi4c    | UMI4C object as generated by <a href="#">makeUMI4C</a> .                                                                    |
| grouping | Grouping used for the different samples. If none available or want to add new groupings, run <a href="#">addGroupings</a> . |
| colors   | Named vector of colors to use for plotting for each group.                                                                  |
| xlim     | Limits for the plot x axis (genomic coordinates).                                                                           |

**Value**

Produces a plot of the fold changes at the differential regions analyzed ghat are contained in the [UMI4C](#) object.

**Examples**

```
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)
```

---

|                 |                         |
|-----------------|-------------------------|
| plotDomainogram | <i>Plot domainogram</i> |
|-----------------|-------------------------|

---

**Description**

Plot domainogram

**Usage**

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

**Arguments**

|                |                                                                                                                                |
|----------------|--------------------------------------------------------------------------------------------------------------------------------|
| umi4c          | UMI4C object as generated by <a href="#">makeUMI4C</a> .                                                                       |
| 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 <a href="#">addGroupings</a> .    |
| colors         | Named vector of colors to use for plotting for each group.                                                                     |
| xlim           | Limits for the plot x axis (genomic coordinates).                                                                              |

**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    | <a href="#">GRanges</a> 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 | <i>Plot interactions</i> |
|------------------|--------------------------|

---

### Description

Plot the results of [callInteractions](#).

### Usage

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

### Arguments

|                 |                                                                                   |
|-----------------|-----------------------------------------------------------------------------------|
| gr_interactions | GRangesList outputed by <a href="#">callInteractions</a> .                        |
| 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 | <i>Plot Interactions UMI4C</i> |
|-----------------------|--------------------------------|

---

### 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
)
```

**Arguments**

|                 |                                                                                                                            |
|-----------------|----------------------------------------------------------------------------------------------------------------------------|
| umi4c           | UMI4C object as generated by <a href="#">makeUMI4C</a> .                                                                   |
| gr_interactions | GRangesList outputed by <a href="#">callInteractions</a> .                                                                 |
| grouping        | Grouping used for the different samples. If none available or want to add new groupings, run <a href="#">addGrouping</a> . |
| 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.

---

|           |                                       |
|-----------|---------------------------------------|
| plotTrend | <i>Plot adaptative smoothen trend</i> |
|-----------|---------------------------------------|

---

**Description**

Plot adaptative smoothen trend

**Usage**

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

**Arguments**

|          |                                                                                                                            |
|----------|----------------------------------------------------------------------------------------------------------------------------|
| umi4c    | UMI4C object as generated by <a href="#">makeUMI4C</a> .                                                                   |
| grouping | Grouping used for the different samples. If none available or want to add new groupings, run <a href="#">addGrouping</a> . |
| 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

*Plot UMI4C data*


---

**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**

|                |                                                                                                                                                                     |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| umi4c          | <a href="#">UMI4C</a> object as generated by <a href="#">makeUMI4C</a> .                                                                                            |
| grouping       | Grouping used for the different samples. If none available or want to add new groupings, run <a href="#">addGrouping</a> .                                          |
| dgram_function | Function used for calculating the fold-change in the domainogram plot, either "difference" or "quotient". Default: "quotient".                                      |
| dgram_plot     | Logical indicating whether to plot the domainogram. If the <a href="#">UMI4C</a> object only contains one sample will be automatically set to FALSE. 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

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

### Examples

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

plotUMI4C(ex_ciita_umi4c,
  dgram_plot = FALSE
)
```

---

|           |                           |
|-----------|---------------------------|
| prepUMI4C | <i>Prepare UMI4C data</i> |
|-----------|---------------------------|

---

### 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

```
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. |

|            |                                                                                                                                       |
|------------|---------------------------------------------------------------------------------------------------------------------------------------|
| 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.                                                                                 |
| 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 log file with the statistics is also generated in `wk_dir/logs` named `umi4c_stats.txt`.

### See Also

[contactsUMI4C](#).

### Examples

```
if (interactive()) {
  path <- downloadUMI4CexampleData(reduced = TRUE)
  raw_dir <- file.path(path, "CIITA", "fastq")

  prepUMI4C(
    fastq_dir = raw_dir,
    wk_dir = file.path(path, "CIITA"),
    bait_seq = "GGACAAGCTCCCTGCAACTCA",
    bait_pad = "GGACTTGCA",
    res_enz = "GATC"
  )
}
```

---

smoothMonotoneUMI4C      *Monotone smoothing of the DDS object 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. 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)
```



**Arguments**

|         |                                                                                                                                                                                                                                                                                              |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| dds     | DDS object as generated by vstUMI4C with the variance stabilized count values.                                                                                                                                                                                                               |
| alpha   | Approximate number of fragments desired for every basis function of the B-spline basis. $\text{floor}((\text{max}(\text{number of fragments})) / \text{alpha})$ is passed to <code>create.bspline.basis</code> as <code>nbasis</code> argument. 4 is the minimum allowed value. Default: 20. |
| penalty | Amount of smoothing to be applied to the estimated functional parameter. Default: 0.1.                                                                                                                                                                                                       |

**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**

|            |                                                                                                                                       |
|------------|---------------------------------------------------------------------------------------------------------------------------------------|
| wk_dir     | Working directory where to save the outputs generated by the UMI-4c analysis.                                                         |
| 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).                  |
| 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. |
| min_flen   | Minimal fragment length to use for selecting the fragments. Default=20                                                                |

**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**

```

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**

```
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**

```

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                                                                                                        | Theme                                                        |
| <b>Description</b>                                                                                           |                                                              |
| Theme                                                                                                        |                                                              |
| <b>Usage</b>                                                                                                 |                                                              |
| theme(...)                                                                                                   |                                                              |
| <b>Arguments</b>                                                                                             |                                                              |
| ...                                                                                                          | Additional arguments to pass to the theme call from ggplot2. |
| <b>Value</b>                                                                                                 |                                                              |
| ggplot2 theme.                                                                                               |                                                              |
| <b>Examples</b>                                                                                              |                                                              |
| <pre>library(ggplot2)  ggplot(   iris,   aes(Sepal.Length, Sepal.Width) ) +   geom_point() +   theme()</pre> |                                                              |
| themeXblank                                                                                                  | Theme X blank                                                |

**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

```
library(ggplot2)

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

---

|              |               |
|--------------|---------------|
| themeXYblank | Theme Y blank |
|--------------|---------------|

---

### 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

```
library(ggplot2)

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

---

|             |                      |
|-------------|----------------------|
| themeYblank | <i>Theme Y blank</i> |
|-------------|----------------------|

---

**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)

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

---

|       |                    |
|-------|--------------------|
| UMI4C | <i>UMI4C-class</i> |
|-------|--------------------|

---

**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_umi4c = NULL,
  bait_exclusion = 3000,
```

```

    bait_expansion = 1e+06,
    scales = 5:150,
    min_win_factor = 0.02,
    sd = 2
)

```

## Arguments

|                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>colData</code>        | Data.frame containing the information for constructing the UMI4C experiment object. Needs to contain the following columns: <ul style="list-style-type: none"> <li>• <code>sampleID</code>. Unique identifier for the sample.</li> <li>• <code>condition</code>. Condition for performing differential analysis. Can be control and treatment, two different cell types, etc.</li> <li>• <code>replicate</code>. Number for identifying replicates.</li> <li>• <code>file</code>. File as outputed by <code>umi4CatsContacts</code> function.</li> </ul> |
| <code>viewpoint_name</code> | Character indicating the name for the used viewpoint.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <code>grouping</code>       | Name of the column in <code>colData</code> used to merge the samples or replicates. Set to <code>NULL</code> for skipping grouping. Default: "condition".                                                                                                                                                                                                                                                                                                                                                                                                |
| <code>normalized</code>     | Logical indicating whether UMI-4C profiles should be normalized to the <code>ref_umi4c</code> sample/group. Default: <code>TRUE</code>                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <code>ref_umi4c</code>      | Name of the sample or group to use as reference for normalization. By default is <code>NULL</code> , 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 <code>colData</code> and the value represents the level to use as reference.                                                                                                                                                                                                          |
| <code>bait_exclusion</code> | Region around the bait (in bp) to be excluded from the analysis. Default: 3000bp.                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <code>bait_expansion</code> | Number of bp upstream and downstream of the bait to use for the analysis (region centered in bait). Default: 1Mb.                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <code>scales</code>         | Numeric vector containing the scales for calculating the domainogram.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <code>min_win_factor</code> | Proportion of UMIs that need to be found in a specific window for adaptative trend calculation                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <code>sd</code>             | Stantard deviation for adaptative trend.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

## Value

It returns an object of the class [UMI4C](#).

## Slots

|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>colData</code> | Data.frame containing the information for constructing the UMI4C experiment object. Needs to contain the following columns: <ul style="list-style-type: none"> <li>• <code>sampleID</code>: Unique identifier for the sample.</li> <li>• <code>condition</code>: Condition for performing differential analysis. Can be control and treatment, two different cell types, etc.</li> <li>• <code>replicate</code>: Number or ID for identifying different replicates.</li> <li>• <code>file</code>: Path to the files outputed by <a href="#">contactsUMI4C</a>.</li> </ul> |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**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 adaptative 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**: Adaptative smoothing trend of UMIs.
4. **geo\_coords**: Geometric coordinates obtained when performing the adaptative smoothing.
5. **scale**: Scale selected for the adaptative smoothing.
6. **sd**: Standard deviation for the adaptative 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 <a href="#">DESeqDataSet</a> . |

## Value

DDS object.



## 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](#).

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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 inferring 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**

|                |                                                                                                                                                                                              |
|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| umi4c          | UMI4C object as generated by makeUMI4C or the UMI4C constructor.                                                                                                                             |
| query_regions  | GRanges object containing the coordinates of the genomic regions you want to use to perform the analysis in specific genomic intervals. Default: NULL.                                       |
| subset         | If query_regions are provided, how to subset the UMI4C object: "sum" for summing raw UMIs in fragments overlapping query_regions (default) or "overlap" for selecting overlapping fragments. |
| design         | A formula or matrix. The formula expresses how the counts for each fragment end depend on the variables in colData. See <a href="#">DESeqDataSet</a> .                                       |
| 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 <a href="#">p.adjust</a> . 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, which can be accessed using [resultsUMI4C](#).

**Examples**

```
data("ex_ciita_umi4c")

umi_dif <- waldUMI4C(ex_ciita_umi4c)
```

---

zscoreUMI4C

*Z-score calculation using residuals of trend and fit UMI4C counts*


---

**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**

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

**Arguments**

|                               |                                                                                                                     |
|-------------------------------|---------------------------------------------------------------------------------------------------------------------|
| <code>dds</code>              | DDS object as generated by <code>smoothMonotoneUMI4C</code> with the smooth monotone fit counts                     |
| <code>padj_method</code>      | The method to use for adjusting p-values, see <a href="#">p.adjust</a> . Default: <code>fdr</code> .                |
| <code>zscore_threshold</code> | Numeric indicating the z-score threshold to use to define significant differential contacts. Default: 2.            |
| <code>padj_threshold</code>   | 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**

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

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