

# Package ‘crisprDesign’

December 1, 2023

**Title** Comprehensive design of CRISPR gRNAs for nucleases and base editors

**Version** 1.4.0

**Description** Provides a comprehensive suite of functions to design and annotate CRISPR guide RNA (gRNAs) sequences. This includes on- and off-target search, on-target efficiency scoring, off-target scoring, full gene and TSS contextual annotations, and SNP annotation (human only). It currently supports five types of CRISPR modalities (modes of perturbations): CRISPR knockout, CRISPR activation, CRISPR inhibition, CRISPR base editing, and CRISPR knockdown. All types of CRISPR nucleases are supported, including DNA- and RNA-target nucleases such as Cas9, Cas12a, and Cas13d. All types of base editors are also supported. gRNA design can be performed on reference genomes, transcriptomes, and custom DNA and RNA sequences. Both unpaired and paired gRNA designs are enabled.

**Depends** R (>= 4.2.0), crisprBase (>= 1.1.3)

**Imports** AnnotationDbi, BiocGenerics, Biostrings, BSgenome, crisprBowtie (>= 0.99.8), crisprScore (>= 1.1.6), GenomeInfoDb, GenomicFeatures, GenomicRanges (>= 1.38.0), IRanges, Matrix, MatrixGenerics, methods, rtracklayer, S4Vectors, stats, utils, VariantAnnotation

**Suggests** biomaRt, BSgenome.Hsapiens.UCSC.hg38, BSgenome.Mmusculus.UCSC.mm10, BiocStyle, crisprBwa (>= 0.99.7), knitr, rmarkdown, Rbowtie, Rbwa, RCurl, testthat

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**Author** Jean-Philippe Fortin [aut, cre],  
Luke Hoberecht [aut]

**Maintainer** Jean-Philippe Fortin <fortin946@gmail.com>

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addCompositeScores      *Add on-target composite score to a [GuideSet](#) object.*

---

### Description

Add on-target composite score to a [GuideSet](#) object.

### Usage

```
addCompositeScores(object,...)

## S4 method for signature 'GuideSet'
addCompositeScores(
  object,
  methods = c("azimuth", "ruleset1", "ruleset3", "lindel", "deepcpf1", "deephf",
    "deepspcas9", "enpamgb", "casrxrf", "crisprater", "crisprscan", "crispra", "crispri"),
  scoreName = "score_composite"
)

## S4 method for signature 'PairedGuideSet'
addCompositeScores(
  object,
```

```

methods = c("azimuth", "ruleset1", "ruleset3", "lindel", "deepcpf1", "deephf",
  "deepspcas9", "enpamgb", "crisprater", "crisprscan", "casrxrf", "crispra", "crispri"),
scoreName = "score_composite"
)

## S4 method for signature ``NULL``
addCompositeScores(object)

```

### Arguments

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
methods	Character vector specifying method names for on-target efficiency prediction algorithms to be used to create the composite score. Note that the specified scores must be added first to the object using <code>addOnTargetScores</code> .
scoreName	String specifying the name of the composite score to be used as a column name. Users can choose whatever they like. Default is "score_composite".

### Details

The function creates a composite score across a specified list of on-target scores by first transforming each individual score into a rank, and then taking the average rank across all specified methods. This can improve on-target activity prediction robustness. A higher score indicates higher on-target activity.

### Value

guideSet with column specified by scoreName appended in `mcols(guideSet)`.

### Author(s)

Jean-Philippe Fortin

### See Also

[addOnTargetScores](#) to add on-target scores.

### Examples

```

gs <- findSpacers("CCAACATAGTGAAACACGCTCTATAAAGAATAAAAAATTAGCCGGGTTA")
gs <- addOnTargetScores(gs, methods=c("ruleset1", "crisprater"))
gs <- addCompositeScores(gs, methods=c("ruleset1", "crisprater"))

```

---

addConservationScores *Add on-target composite score to a [GuideSet](#) object.*

---

## Description

Add on-target composite score to a [GuideSet](#) object.

## Usage

```
addConservationScores(object, ...)  
  
## S4 method for signature 'GuideSet'  
addConservationScores(  
  object,  
  conservationFile,  
  nucExtension = 9,  
  fun = c("mean", "max"),  
  scoreName = "score_conservation"  
)  
  
## S4 method for signature 'PairedGuideSet'  
addConservationScores(  
  object,  
  conservationFile,  
  nucExtension = 9,  
  fun = c("mean", "max"),  
  scoreName = "score_conservation"  
)  
  
## S4 method for signature '`NULL`'  
addConservationScores(object)
```

## Arguments

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
conservationFile	String specifying the BigWig file containing conservation scores.
nucExtension	Number of nucleotides to include on each side of the cut site to calculate the conservation score. 9 by default. The region will have (2*nucExtension + 1) nucleotides in total.
fun	String specifying the function to use to calculate the final conservation score in the targeted region. Must be either "mean" (default) or "max".
scoreName	String specifying the name of the conservation score to be used as a column name. Users can choose whatever they like. Default is "score_conservation".

**Details**

The function creates a conservation score for each gRNA by using the max, or average, conservation score in the genomic region where the cut occurs. A BigWig file storing conservation scores must be provided. Such files can be downloaded from the UCSC genome browser. See vignette for more information.

**Value**

guideSet with column specified by scoreName appended in mcols(guideSet).

**Author(s)**

Jean-Philippe Fortin

---

addCrispraiScores      *Add CRISPRa/CRISPRi on-target scores to a [GuideSet](#) object.*

---

**Description**

Add CRISPRa/CRISPRi on-target scores to a [GuideSet](#) object. Only available for SpCas9, and for hg38 genome. Requires **crisprScore** package to be installed.

**Usage**

```
addCrispraiScores(object, ...)

## S4 method for signature 'GuideSet'
addCrispraiScores(
  object,
  gr,
  tssObject,
  geneCol = "gene_id",
  modality = c("CRISPRi", "CRISPRa"),
  chromatinFiles = NULL,
  fastaFile = NULL
)

## S4 method for signature 'PairedGuideSet'
addCrispraiScores(
  object,
  gr,
  tssObject,
  geneCol = "gene_id",
  modality = c("CRISPRi", "CRISPRa"),
  chromatinFiles = NULL,
  fastaFile = NULL
)
```

```
## S4 method for signature '`NULL`'
addCrispraiScores(object)
```

### Arguments

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
gr	A <a href="#">GRanges</a> object derived from queryTss used to produce the guideSet object.
tssObject	A <a href="#">GRanges</a> object containing TSS coordinates and annotation. The following columns must be present: "ID", promoter", "tx_id" and "gene_symbol".
geneCol	String specifying which column of the tssObject should be used for a unique gene identified. "gene_id" by default.
modality	String specifying which modality is used. Must be either "CRISPRi" or "CRISPRa".
chromatinFiles	Named character vector of length 3 specifying BigWig files containing chromatin accessibility data. See <a href="#">crisprScore</a> vignette for more information.
fastaFile	String specifying fasta file of the hg38 genome.

### Value

guideSet with an added column for the CRISPRai score.

### Author(s)

Jean-Philippe Fortin

### See Also

[addOnTargetScores](#) to add other on-target scores.

---

addDistanceToTss	<i>Add distance to TSS for a specified TSS id</i>
------------------	---

---

### Description

Add distance to TSS for a specified TSS id.

### Usage

```
addDistanceToTss(object, ...)
```

```
## S4 method for signature 'GuideSet'
addDistanceToTss(object, tss_id)
```

```
## S4 method for signature 'PairedGuideSet'
addDistanceToTss(object, tss_id)
```

**Arguments**

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
tss_id	String specifying TSS id to calculate the distance. The column <code>tssAnnotation(object)\$tss_id</code> will be used to search for the TSS id.

**Value**

A [GuideSet](#) object or a [PairedGuideSet](#) object with an additional metadata column called `distance_to_tss` reporting the distance (in nucleotides) between the TSS position of the TSS specified by `tss_id` and the protospacer position. The `pam_site` coordinate is used as the representative position of protospacer sequences.

Note that a TSS annotation must be available in the object. A TSS annotation can be added using `addTssAnnotation`.

**Author(s)**

Jean-Philippe Fortin

**See Also**

[addTssAnnotation](#) to add TSS annotation.

**Examples**

```
data(guideSetExampleFullAnnotation)
tss_id <- "ENSG00000120645_P1"
gs <- guideSetExampleFullAnnotation
gs <- addDistanceToTss(gs, tss_id)
```

---

`addEditedAlleles`      *To add edited alleles for a CRISPR base editing GuideSet*

---

**Description**

To add edited alleles for a CRISPR base editing GuideSet.

**Usage**

```
addEditedAlleles(
  guideSet,
  baseEditor,
  editingWindow = NULL,
  nMaxAlleles = 100,
  addFunctionalConsequence = TRUE,
  addSummary = TRUE,
```



```

    txTable = NULL,
    verbose = TRUE
  )

```

### Arguments

guideSet	A <a href="#">GuideSet</a> object.
baseEditor	A <a href="#">BaseEditor</a> object.
editingWindow	A numeric vector of length 2 specifying start and end positions of the editing window with respect to the PAM site. If NULL (default), the editing window of the BaseEditor object will be considered.
nMaxAlleles	Maximum number of edited alleles to report for each gRNA. Alleles from high to low scores. 100 by default.
addFunctionalConsequence	Should variant classification of the edited alleles be added? TRUE by default. If TRUE, txTable must be provided.
addSummary	Should a summary of the variant classified by added to the metadata columns of the guideSet object? TRUE by default.
txTable	Table of transcript-level nucleotide and amino acid information needed for variant classification. Usually returned by <a href="#">getTxInfoDataFrame</a> .
verbose	Should messages be printed to console? TRUE by default.

### Value

The original guideSet object with an additional metadata column (editedAlleles) storing the annotated edited alleles. The edited alleles are always reported from 5' to 3' direction on the strand corresponding to the gRNA strand.

### Author(s)

Jean-Philippe Fortin

### Examples

```

data(BE4max, package="crisprBase")
data(grListExample, package="crisprDesign")
library(BSgenome.Hsapiens.UCSC.hg38)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38
gr <- queryTxObject(grListExample,
                    featureType="cds",
                    queryColumn="gene_symbol",
                    queryValue="IQSEC3")
gs <- findSpacers(gr[1],
                 crisprNuclease=BE4max,
                 bsgenome=bsgenome)
gs <- unique(gs)
gs <- gs[1:2] # For the sake of time

# Getting transcript info:

```

```

txid="ENST00000538872"
txTable <- getTxInfoDataFrame(tx_id=txid,
  txObject=grListExample,
  bsgenome=bsgenome)

#Adding alleles:
editingWindow <- c(-20,-8)
gs <- addEditedAlleles(gs,
  baseEditor=BE4max,
  txTable=txTable,
  editingWindow=editingWindow)

```

---

addEditingSites	<i>Add optimal editing site for base editing gRNAs.</i>
-----------------	---

---

### Description

Add optimal editing site for base editing gRNAs.

### Usage

```

addEditingSites(object, ...)

## S4 method for signature 'GuideSet'
addEditingSites(object, substitution)

## S4 method for signature 'PairedGuideSet'
addEditingSites(object, substitution)

## S4 method for signature '`NULL`'
addEditingSites(object)

```

### Arguments

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
substitution	String indicating which substitution should be used to estimate the optimal editing position. E.g. "C2T" will return the optimal editing position for C to T editing.

### Value

An updated object with a column `editing_site` added to `mcols(object)`.

### Author(s)

Jean-Philippe Fortin

---

addExonTable	<i>Add a gene-specific exon table to a <a href="#">GuideSet</a> object.</i>
--------------	---

---

### Description

Add a gene-specific exon table to a [GuideSet](#) object.

Add a gene-specific exon table to a [GuideSet](#) object.

### Usage

```
addExonTable(  
  guideSet,  
  gene_id,  
  txObject,  
  valueColumn = "percentCDS",  
  useConsensusIsoform = FALSE  
)
```

### Arguments

guideSet	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
gene_id	String specifying gene ID.
txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained using <a href="#">TxDb2GRangesList</a> to provide a gene model annotation.
valueColumn	String specifying column in <code>geneAnnotation(guideSet)</code> to use as values in the output exon table.
useConsensusIsoform	Should a consensus isoform be used to annotate exons? FALSE by default. If TRUE, the isoform constructed by <code>getConsensusIsoform</code> will be used.

### Value

A [GuideSet](#) object with a "exonTable" `DataFrame` stored in `mcols(guideSet)`. The entries in the `DataFrame` correspond to the values specified by `valueColumn`. Rows correspond to gRNAs in the `GuideSet`, columns correspond to all exons found in `txObject` for gene specified by `gene_id`.

### Author(s)

Jean-Philippe Fortin

### See Also

[addGeneAnnotation](#) to add gene annotation and [addTxTable](#) to add a transcript table.

**Examples**

```

if (interactive()){
  data(guideSetExample, package="crisprDesign")
  data(grListExample, package="crisprDesign")
  guideSet <- addGeneAnnotation(guideSetExample,
                              txObject=grListExample)
  guideSet <- addExonTable(guideSet,
                          gene_id="ENSG00000120645",
                          txObject=grListExample)

  guideSet$exonTable
}

```

---

addGeneAnnotation      *Add gene context annotation to a [GuideSet](#) object*

---

**Description**

Add gene context annotation to spacer sequence stored in a [GuideSet](#) object

**Usage**

```

addGeneAnnotation(object, ...)

## S4 method for signature 'GuideSet'
addGeneAnnotation(
  object,
  txObject,
  anchor = c("cut_site", "pam_site", "editing_site"),
  ignore_introns = TRUE,
  ignore.strand = TRUE,
  addPfam = FALSE,
  mart_dataset = NULL
)

## S4 method for signature 'PairedGuideSet'
addGeneAnnotation(
  object,
  txObject,
  anchor = c("cut_site", "pam_site", "editing_site"),
  ignore_introns = TRUE,
  ignore.strand = TRUE,
  addPfam = FALSE,
  mart_dataset = NULL
)

```

**Arguments**

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained using <a href="#">TxDb2GRangesList</a> to provide a gene model annotation.
anchor	String specifying which relative coordinate of gRNAs should be used to locate gRNAs within gene. Must be either "cut_site", "pam_site" or "editing_site".
ignore_introns	Should gene introns be ignored when annotating? TRUE by default.
ignore_strand	Should gene strand be ignored when annotating? TRUE by default.
addPfam	Should Pfam domains annotation be added? FALSE by default. If set to TRUE, <b>biomaRt</b> must be installed.
mart_dataset	String specifying dataset to be used by <b>biomaRt</b> for Pfam domains annotation . E.g. "hsapiens_gene_ensembl".

**Details**

For DNA-targeting nucleases, the different columns stored in `mcols(guideSet)[["geneAnnotation"]]` are:

- tx\_id Transcript ID.
- gene\_symbol Gene symbol.
- gene\_id Gene ID.
- protein\_id Protein ID.
- ID gRNA ID.
- pam\_site gRNA PAM site coordinate.
- cut\_site gRNA cut site coordinate.
- chr gRNA chromosome name.
- strand gRNA strand.
- cut\_cds Is the gRNA cut site located within the coding sequence (CDS) of the targeted isoform?
- cut\_fiveUTRs Is the gRNA cut site located within the 5'UTR of the targeted isoform?
- cut\_threeUTRs Is the gRNA cut site located within the 3'UTR of the targeted isoform?
- cut\_introns Is the gRNA cut site located within an intron of the targeted isoform?
- percentCDS Numeric value to indicate the relative position of the cut site with respect to the start of the CDS sequence when cut\_cds is TRUE. The relative position is expressed as a percentage from the total length of the CDS.
- percentTx Numeric value to indicate the relative position of the cut site with respect to the start of the mRNA sequence (therefore including 5' UTR). The relative position is expressed as a percentage from the total length of the mRNA sequence.
- aminoAcidIndex If cut\_cds is TRUE, integer value indicating the amino acid index with respect to the start of the protein.

- `downstreamATG` Numeric value indicating the number of potential reinitiation sites (ATG codons) downstream of the gRNA cut site, within 250 amino acids.
- `nIsoforms` Numeric value indicating the number of isoforms targeted by the gRNA.
- `totalIsoforms` Numeric value indicating the total number of isoforms existing for the gene targeted by the gRNA and specified in `gene_id`.
- `percentIsoforms` Numeric value indicating the percentage of isoforms for the gene specified in `gene_id` targeted by the gRNA. Equivalent to  $nIsoforms/totalIsoforms*100$ .
- `isCommonExon` Logical value to indicate whether or not the gRNA is targeting an exon common to all isoforms.
- `nCodingIsoforms` Numeric value indicating the number of coding isoforms targeted by the gRNA. 5' UTRs and 3' UTRs are excluded.
- `totalCodingIsoforms` Numeric value indicating the total number of coding isoforms existing for the gene targeted by the gRNA and specified in `gene_id`.
- `percentCodingIsoforms` Numeric value indicating the percentage of coding isoforms for the gene specified in `gene_id` targeted by the gRNA. Equivalent to  $nCodingIsoforms/totalCodingIsoforms*100$ . 5' UTRs and 3' UTRs are excluded.
- `isCommonCodingExon` Logical value to indicate whether or not the gRNA is targeting an exon common to all coding isoforms.

### Value

A [GuideSet](#) object with a "geneAnnotation" list column stored in `mcols(guideSet)`. See details section for a description of the different gene annotation columns.

### Author(s)

Jean-Philippe Fortin, Luke Hoberecht

### See Also

[addTssAnnotation](#) to add TSS annotation, and [geneAnnotation](#) to retrieve an existing gene annotation.

### Examples

```
data(guideSetExample, package="crisprDesign")
data(grListExample, package="crisprDesign")
guideSet <- addGeneAnnotation(guideSetExample[1:6],
                             txObject=grListExample)

# To access a gene annotation already added:
ann <- geneAnnotation(guideSet)
```

---

addIsoformAnnotation *Add isoform-specific annotation to a [GuideSet](#) object*

---

## Description

Add isoform-specific annotation to a [GuideSet](#) object.

## Usage

```
addIsoformAnnotation(object, ...)  
  
## S4 method for signature '`NULL`'  
addDistanceToTss(object)  
  
## S4 method for signature 'GuideSet'  
addIsoformAnnotation(object, tx_id)  
  
## S4 method for signature 'PairedGuideSet'  
addIsoformAnnotation(object, tx_id)  
  
## S4 method for signature '`NULL`'  
addIsoformAnnotation(object)
```

## Arguments

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
tx_id	String specifying Ensembl ID for the isoform transcript of interested. E.g. "ENST00000311936".

## Value

A [GuideSet](#) object or a [PairedGuideSet](#) object with the following added columns: percentCDS, percentCodingIsoforms, and isCommonCodingExon. The column values are specific to the transcript specified by tx\_id. The percentCDS column indicates at what percentage of the coding sequence the gRNA is cutting. The column percentCodingIsoforms indicates the percentage of coding isoforms that are targeted by the gRNA. The column isCommonCodingExon indicates whether or not the exon targeted by the gRNA is common to all isoforms for the gene.

## Author(s)

Jean-Philippe Fortin

**Examples**

```
data(guideSetExampleFullAnnotation)
tx_id <- "ENST00000538872"
gs <- guideSetExampleFullAnnotation
gs <- addIsoformAnnotation(gs, tx_id)
```

---

addNtcs

*Add non-targeting control (NTC) sequences to [GuideSet](#)*


---

**Description**

Add non-targeting control (NTC) sequences to a [GuideSet](#) object.

**Usage**

```
addNtcs(object, ...)

## S4 method for signature 'GuideSet'
addNtcs(object, ntcs)

## S4 method for signature 'PairedGuideSet'
addNtcs(object, ntcs)

## S4 method for signature '`NULL`'
addNtcs(object, ...)
```

**Arguments**

object	A <a href="#">GuideSet</a> or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
ntcs	A named character vector of NTC sequences. Sequences must consist of appropriate DNA or RNA bases, and have the same spacer length as spacers in object. Vector names are assigned as IDs and seqlevels, and must be unique and distinct from IDs and seqnames present in object.

**Details**

NTC sequences are appended as spacers to the [GuideSet](#) object. Each NTC sequence is assigned to its own "chromosome" in the ntc genome, as reflected in the [Seqinfo](#) of the resulting [GuideSet](#) object. As placeholder values, NTC ranges are set to 0 and strands set to \*.

All annotation for NTC spacers appended to object are set to NA or empty list elements. To annotate NTC spacers, you must call the appropriate function after adding NTCs to the [GuideSet](#) object.

**Value**

The original object with appended ntcs spacers. Pre-existing annotation in object will be set to NA or empty list elements for appended NTC spacers.



**Examples**

```

set.seed(1000)
data(guideSetExample, package="crisprDesign")
ntcs <- vapply(1:4, function(x){
  seq <- sample(c("A", "C", "G", "T"), 20, replace=TRUE)
  paste0(seq, collapse="")
}, FUN.VALUE=character(1))
names(ntcs) <- paste0("ntc_", 1:4)
gs <- addNtcs(guideSetExample, ntcs)
gs

```

---

addOffTargetScores      *Add CFD and MIT scores to a [GuideSet](#) object.*

---

**Description**

Add CFD and MIT off-target scores to a [GuideSet](#) object. Both the CFD and MIT methods are available for the SpCas9 nuclease. The CFD method is also available for the CasRx nuclease. Other nucleases are currently not supported.

**Usage**

```

addOffTargetScores(object, ...)

## S4 method for signature 'GuideSet'
addOffTargetScores(object, max_mm = 2, includeDistance = TRUE, offset = 0)

## S4 method for signature 'PairedGuideSet'
addOffTargetScores(object, max_mm = 2, includeDistance = TRUE, offset = 0)

## S4 method for signature ``NULL``
addOffTargetScores(object)

```

**Arguments**

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object. <code>crisprNuclease(object)</code> must be either using SpCas9 or CasRx.
...	Additional arguments, currently ignored.
max_mm	The maximum number of mismatches between the spacer sequence and the protospacer off-target sequence to be considered in the off-target score calculations. Off-targets with a number of mismatches greater than <code>max_mm</code> will be excluded; this is useful if one wants to avoid the aggregated off-target scores to be driven by a large number of off-targets that have low probability of cutting.
includeDistance	Should a distance penalty for the MIT score be included? TRUE by default.

offset            Numeric value specifying an offset to add to the denominator when calculating the aggregated score (inverse summation formula). 0 by default.

### Details

See the **crisprScore** package for a description of the different off-target scoring methods.

### Value

A GuideSet or a PairedGuideSet object with added scores. The alignments annotation returned by `alignments(object)` will have additional column storing off-target scores. Those scores representing the off-target score for each gRNA and off-target pair. For SpCas9, a column containing an aggregated specificity off-target score for each scoring method is added to the metadata columns obtained by `mcols(object)`.

### Author(s)

Jean-Philippe Fortin, Luke Hoberecht

### See Also

`link{addOnTargetScores}` to add on-target scores.

### Examples

```
data(guideSetExampleWithAlignments, package="crisprDesign")
gs <- guideSetExampleWithAlignments
gs <- addOffTargetScores(gs)
```

---

`addOnTargetScores`      *Add on-target scores to a [GuideSet](#) object.*

---

### Description

Add on-target scores to a [GuideSet](#) object for all methods available in the **crisprScore** package for a given CRISPR nuclease. Requires **crisprScore** package to be installed.

### Usage

```
addOnTargetScores(object, ...)

## S4 method for signature 'GuideSet'
addOnTargetScores(
  object,
  enzyme = c("WT", "ESP", "HF"),
  promoter = c("U6", "T7"),
  tracrRNA = c("Hsu2013", "Chen2013"),
```

```

    directRepeat = "aaccctaccaactggtcggggtttgaaac",
    binaries = NULL,
    methods = c("azimuth", "ruleset1", "ruleset3", "lindel", "deepcpf1", "deephf",
               "deepspcas9", "enpamgb", "casrxrf", "crisprater", "crisprscan")
  )

## S4 method for signature 'PairedGuideSet'
addOnTargetScores(
  object,
  enzyme = c("WT", "ESP", "HF"),
  promoter = c("U6", "T7"),
  tracrRNA = c("Hsu2013", "Chen2013"),
  directRepeat = "aaccctaccaactggtcggggtttgaaac",
  binaries = NULL,
  methods = c("azimuth", "ruleset1", "ruleset3", "lindel", "deepcpf1", "deephf",
             "deepspcas9", "enpamgb", "crisprater", "crisprscan", "casrxrf")
)

## S4 method for signature ``NULL``
addOnTargetScores(object)

```

## Arguments

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
enzyme	Character string specifying the Cas9 variant to be used for DeepHF scoring. Wildtype Cas9 (WT) by default. See details below.
promoter	Character string specifying promoter used for expressing sgRNAs for wildtype Cas9 (must be either "U6" or "T7") for DeepHF scoring. "U6" by default.
tracrRNA	String specifying which tracrRNA is used for SpCas9. Must be either "Hsu2013" (default) or "Chen2013". Only used for the RuleSet3 method.
directRepeat	String specifying the direct repeat used in the CasRx construct.
binaries	Named list of paths for binaries needed for CasRx-RF. Names of the list must be "RNAfold", "RNAhybrid", and "RNAplfold". Each list element is a string specifying the path of the binary. If NULL (default), binaries must be available on the PATH.
methods	Character vector specifying method names for on-target efficiency prediction algorithms.

## Details

See [crisprScore](#) package for a description of each score.

## Value

guideSet with columns of on-target scores appended in `mcols(guideSet)`.

**Author(s)**

Jean-Philippe Fortin, Luke Hoberecht

**See Also**

[addOffTargetScores](#) to add off-target scores.

**Examples**

```
if (interactive()){  
  gs <- findSpacers("CCAACATAGTGAAACCACGTCTCTATAAAGAATAAAAAATTAGCCGGGTTA")  
  gs <- addOnTargetScores(gs)  
}
```

---

addOpsBarcodes	<i>Add optical pooled screening (OPS) barcodes</i>
----------------	--

---

**Description**

Add optical pooled screening (OPS) barcodes.

**Usage**

```
addOpsBarcodes(guideSet, n_cycles = 9, rt_direction = c("5prime", "3prime"))
```

**Arguments**

guideSet	A <a href="#">GuideSet</a> object.
n_cycles	Integer specifying the number of sequencing cycles used in the in situ sequencing. This effectively determines the length of the barcodes to be used for sequencing.
rt_direction	String specifying from which direction the reverse transcription of the gRNA spacer sequence will occur. Must be either "5prime" or "3prime". "5prime" by default.

**Value**

The original guideSet object with an additional column opsBarcode stored in `mcols(guideSet)`. The column is a `DNAStrngSet` storing the OPS barcode.

**Author(s)**

Jean-Philippe Fortin

## Examples

```
data(guideSetExample, package="crisprDesign")
guideSetExample <- addOpsBarcodes(guideSetExample)
```

---

addPamScores	<i>Add PAM scores to a <a href="#">GuideSet</a> object.</i>
--------------	---

---

## Description

Add PAM scores to a [GuideSet](#) object based on the [CrisprNuclease](#) object stored in the [GuideSet](#) object. PAM scores indicate nuclease affinity (recognition) to different PAM sequences. A score of 1 indicates a PAM sequence that is fully recognized by the nuclease.

## Usage

```
addPamScores(object, ...)
```

## S4 method for signature 'GuideSet'

```
addPamScores(object)
```

## S4 method for signature 'PairedGuideSet'

```
addPamScores(object)
```

## S4 method for signature ``NULL``

```
addPamScores(object)
```

## Arguments

object	A <a href="#">GuideSet</a> or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.

## Value

guideSet with an appended score\_pam column in `mcols(guideSet)`.

## Author(s)

Jean-Philippe Fortin

## Examples

```
# Using character vector as input:
data(enAsCas12a, package="crisprBase")
gs <- findSpacers("CCAACATAGTGAAACCGTCTCTATAAAGAATACAAAAAATTAGCCGGGTGTTA",
                 canonical=FALSE,
                 crisprNuclease=enAsCas12a)
gs <- addPamScores(gs)
```

---

addPfamDomains	<i>Add Pfam domains annotation to <a href="#">GuideSet</a> object</i>
----------------	---

---

### Description

Add Pfam domains annotation to [GuideSet](#) object.

### Usage

```
addPfamDomains(object, ...)  
  
## S4 method for signature 'GuideSet'  
addPfamDomains(object, pfamTable)  
  
## S4 method for signature 'PairedGuideSet'  
addPfamDomains(object, pfamTable)  
  
## S4 method for signature '`NULL`'  
addPfamDomains(object)
```

### Arguments

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
pfamTable	A <a href="#">DataFrame</a> obtained using <a href="#">preparePfamTable</a> .

### Details

In order to call this function, the object must contain a gene annotation by calling first [addGeneAnnotation](#).

### Value

An updated object with a column pfam added to `geneAnnotation(object)`.

### Author(s)

Jean-Philippe Fortin

### See Also

See [preparePfamTable](#) to prepare the Pfam domain DataFrame object, and see [addGeneAnnotation](#) to add a gene annotation to the object.

---

addRepeats	Annotate a <i>GuideSet</i> object with repeat elements
------------	--

---

### Description

Add an annotation column to a *GuideSet* object that identifies spacer sequences overlapping repeat elements.

### Usage

```
addRepeats(object, ...)  
  
## S4 method for signature 'GuideSet'  
addRepeats(object, gr.repeats = NULL, ignore.strand = TRUE)  
  
## S4 method for signature 'PairedGuideSet'  
addRepeats(object, gr.repeats = NULL, ignore.strand = TRUE)  
  
## S4 method for signature ``NULL``  
addRepeats(object)
```

### Arguments

object	A <i>GuideSet</i> object or a <i>PairedGuideSet</i> object.
...	Additional arguments, currently ignored.
gr.repeats	A <i>GRanges</i> object containing repeat elements regions.
ignore.strand	Should gene strand be ignored when annotating? TRUE by default.

### Value

*guideSet* with an *inRepeats* column appended in *mcols(guideSet)* that signifies whether the spacer sequence overlaps a repeat element.

### Author(s)

Jean-Philippe Fortin, Luke Hoberecht

### See Also

[link{removeRepeats}](#).

### Examples

```
data(guideSetExample, package="crisprDesign")  
data(grRepeatsExample, package="crisprDesign")  
guideSet <- addRepeats(guideSetExample,  
                       gr.repeats=grRepeatsExample)
```

---

addRestrictionEnzymes *Restriction enzyme recognition sites in spacer sequences*

---

## Description

Add restriction site enzymes annotation.

## Usage

```
addRestrictionEnzymes(object, ...)

## S4 method for signature 'GuideSet'
addRestrictionEnzymes(
  object,
  enzymeNames = NULL,
  patterns = NULL,
  includeDefault = TRUE,
  flanking5 = "ACCG",
  flanking3 = "GTTT"
)

## S4 method for signature 'PairedGuideSet'
addRestrictionEnzymes(
  object,
  enzymeNames = NULL,
  patterns = NULL,
  includeDefault = TRUE,
  flanking5 = "ACCG",
  flanking3 = "GTTT"
)

## S4 method for signature ``NULL``
addRestrictionEnzymes(object)
```

## Arguments

object	A <a href="#">GuideSet</a> or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
enzymeNames	Character vector of enzyme names.
patterns	Optional named character vector for custom restriction site patterns. Vector names are treated as enzymes names. See example.
includeDefault	Should commonly-used enzymes be included? TRUE by default.
flanking5, flanking3	Character string indicating the 5' or 3' flanking sequence, respectively, of the spacer sequence in the lentiviral vector.





---

addSequenceFeatures    *Add spacer sequence feature annotation columns to a [GuideSet](#) object*

---

## Description

Add spacer sequence feature annotation columns, such as GC content, homopolymers, and hairpin predictions, to a [GuideSet](#) object.

## Usage

```
addSequenceFeatures(object, ...)  
  
## S4 method for signature 'GuideSet'  
addSequenceFeatures(  
  object,  
  addHairpin = FALSE,  
  backbone = "AGGCTAGTCCGT",  
  tp53 = TRUE,  
  ...  
)  
  
## S4 method for signature 'PairedGuideSet'  
addSequenceFeatures(  
  object,  
  addHairpin = FALSE,  
  backbone = "AGGCTAGTCCGT",  
  tp53 = TRUE,  
  ...  
)  
  
## S4 method for signature '`NULL`'  
addSequenceFeatures(object, ...)
```

## Arguments

object	A <a href="#">GuideSet</a> or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
addHairpin	Whether to include predicted hairpin formation via sequence complementarity. FALSE by default. See details.
backbone	Backbone sequence in the guide RNA that is susceptible to hairpin formation with a complementary region in the spacer sequence.
tp53	Should TP53-related toxicity features be added? TRUE by default. See details.

## Details

The `addHairpin` argument set to `TRUE` will indicate which spacers are predicted to form internal hairpins. Such hairpins can happen when there is a palindromic sequence within the spacer having arms of  $\geq 4$ nt and  $\geq 50\%$  GC content, and are separated by a loop of  $\geq 4$ nt. Backbone hairpin formation is predicted when the spacer and backbone share a complementary sequence of  $\geq 5$ nt and  $\geq 50\%$  GC content. The argument `backbone` allows users to specify the vector backbone sequence directly downstream of the spacer sequence.

The `tp53` argument set to `TRUE` will add sequence-based features that have been reported to make SpCas9 gRNAs toxic for cells with wildtype TP53 (see <https://doi.org/10.1038/s41467-022-32285-1>). Currently, only one feature is reported and consists of the extended NNGG PAM sequence (1 nucleotide + PAM sequence) for SpCas9. gRNAs with extended CNGG PAM sequences, and in particular CCGG, should be avoided.

## Value

The original object with the following columns appended to `mcols(object)`:

- `percentGC` — percent GC content
- `polyA`, `polyC`, `polyG`, `polyT` — presence of homopolymers of 4nt or longer
- `selfHairpin` — prediction of hairpin formation within the spacer sequence via self-complementarity if `addHairpin` is `TRUE`.
- `backboneHairpin` — prediction of hairpin formation with the backbone sequence via complementarity if `addHairpin` is `TRUE`.
- `NNGG` — extended PAM sequence for SpCas9 if `tp53` is `TRUE` corresponding to one nucleotide upstream of the PAM sequence followed by the PAM sequence itself.

## Examples

```
custom_seq <- c("ATTTCCGGAGGCGGAGAGGCGGGAGGAGCG")
data(SpCas9, package="crisprBase")
guideSet <- findSpacers(custom_seq, crisprNuclease=SpCas9)
guideSet <- addSequenceFeatures(guideSet)
```

---

<code>addSNPAnnotation</code>	<i>Add SNP annotation to a <a href="#">GuideSet</a> object</i>
-------------------------------	--

---

## Description

Add SNP annotation to a [GuideSet](#) object. Only available for sgRNAs designed for human genome.

**Usage**

```
addSNPAnnotation(object, ...)

## S4 method for signature ``NULL``
addGeneAnnotation(object)

## S4 method for signature 'GuideSet'
addSNPAnnotation(object, vcf, maf = 0.01)

## S4 method for signature 'PairedGuideSet'
addSNPAnnotation(object, vcf, maf = 0.01)

## S4 method for signature ``NULL``
addSNPAnnotation(object)
```

**Arguments**

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
vcf	Either a character string specifying a path to a VCF file or connection, or a <a href="#">VCF</a> object.
maf	Minimum minor allele frequency to report (for a least one source among 1000Genomes and TOPMED). Must be between 0 and 1 (exclusive).

**Details**

The different columns stored in `mcols(guideSet)[["snps"]]` are:

- ID sgRNA ID.
- rs Reference SNP cluster ID (e.g. rs17852242)
- rs\_site Genomic coordinate of the SNP.
- rs\_site\_rel Position of SNP relative to the PAM site.
- allele\_ref DNASTring specifying the SNP reference allele.
- allele\_minor DNASTring specifying the SNP minor allele.
- MAF\_1000G Minor allele frequency in the 1000 Genomes project.
- MAF\_TOPMED Minor allele frequency in the TOPMed project.
- type Type of SNP ("ins": insertion, "del": deletion).
- length Length of SNP in nucleotides.

**Value**

guideSet appended with hasSNP column and snps list-column, both stored in `mcols{guideSet}`.

**See Also**

`link{snpAnnotation}` to retrieve an existing SNP annotation stored in a [GuideSet](#) object. See details section for a description of the different columns.

**Examples**

```
vcf <- system.file("extdata",
                  file="common_snps_dbsnp151_example.vcf.gz",
                  package="crisprDesign")
data(guideSetExample, package="crisprDesign")
guideSet <- addSNPAnnotation(guideSetExample, vcf=vcf)
```

---

addSpacerAlignments     *Functions for finding and characterizing on- and off-targets of spacer sequences.*

---

**Description**

Functions for finding and characterizing on- and off-targets of spacer sequences.

**Usage**

```
addSpacerAlignments(object, ...)

addSpacerAlignmentsIterative(object, ...)

## S4 method for signature 'GuideSet'
addSpacerAlignmentsIterative(
  object,
  aligner = c("bowtie", "bwa", "biostrings"),
  colname = "alignments",
  addSummary = TRUE,
  txObject = NULL,
  tssObject = NULL,
  custom_seq = NULL,
  aligner_index = NULL,
  bsgenome = NULL,
  n_mismatches = 0,
  all_alignments = FALSE,
  canonical = TRUE,
  standard_chr_only = FALSE,
  both_strands = TRUE,
  anchor = c("cut_site", "pam_site"),
  annotationType = c("gene_symbol", "gene_id"),
  tss_window = NULL,
  alignmentThresholds = c(n0 = 5, n1 = 100, n2 = 100, n3 = 1000, n4 = 1000)
)

## S4 method for signature 'PairedGuideSet'
addSpacerAlignmentsIterative(
  object,
```

```
aligner = c("bowtie", "bwa", "biostrings"),
colname = "alignments",
addSummary = TRUE,
txObject = NULL,
tssObject = NULL,
custom_seq = NULL,
aligner_index = NULL,
bsgenome = NULL,
n_mismatches = 0,
all_alignments = FALSE,
canonical = TRUE,
standard_chr_only = FALSE,
both_strands = TRUE,
anchor = c("cut_site", "pam_site"),
annotationType = c("gene_symbol", "gene_id"),
tss_window = NULL,
alignmentThresholds = c(n0 = 5, n1 = 100, n2 = 100, n3 = 1000, n4 = 1000)
)

## S4 method for signature ``NULL``
addSpacerAlignmentsIterative(object)

## S4 method for signature 'GuideSet'
addSpacerAlignments(
  object,
  aligner = c("bowtie", "bwa", "biostrings"),
  colname = "alignments",
  addSummary = TRUE,
  txObject = NULL,
  tssObject = NULL,
  custom_seq = NULL,
  aligner_index = NULL,
  bsgenome = NULL,
  n_mismatches = 0,
  n_max_alignments = 1000,
  all_alignments = TRUE,
  canonical = TRUE,
  standard_chr_only = FALSE,
  both_strands = TRUE,
  anchor = c("cut_site", "pam_site"),
  annotationType = c("gene_symbol", "gene_id"),
  tss_window = NULL
)

## S4 method for signature 'PairedGuideSet'
addSpacerAlignments(
  object,
  aligner = c("bowtie", "bwa", "biostrings"),
```

```

    colname = "alignments",
    addSummary = TRUE,
    txObject = NULL,
    tssObject = NULL,
    custom_seq = NULL,
    aligner_index = NULL,
    bsgenome = NULL,
    n_mismatches = 0,
    n_max_alignments = 1000,
    all_alignments = FALSE,
    canonical = TRUE,
    standard_chr_only = FALSE,
    both_strands = TRUE,
    anchor = c("cut_site", "pam_site"),
    annotationType = c("gene_symbol", "gene_id"),
    tss_window = NULL
)

## S4 method for signature ``NULL``
addSpacerAlignments(object)

getSpacerAlignments(
  spacers,
  aligner = c("bowtie", "bwa", "biostrings"),
  custom_seq = NULL,
  aligner_index = NULL,
  bsgenome = NULL,
  n_mismatches = 0,
  n_max_alignments = 1000,
  all_alignments = TRUE,
  crisprNuclease = NULL,
  canonical = TRUE,
  standard_chr_only = FALSE,
  both_strands = TRUE
)

```

### Arguments

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
aligner	Which genomic alignment method should be used? Must be one of "bowtie", "bwa", and "biostrings". "bowtie" by default. Note that "bwa" is not available for Windows machines.
colname	String specifying the column name storing the alignments in <code>mcols(guideSet)</code> . "alignments" by default.
addSummary	Should summary columns be added to <code>guideSet</code> ? TRUE by default.
txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained using <a href="#">TxDb2GRangesList</a> for annotating on-target and off-target alignments using gene annotation.

tssObject	A <a href="#">GRanges</a> object specifying TSS coordinates.
custom_seq	Optional string specifying the target DNA sequence for the search space. This will limit the off-target search to the specified custom sequence.
aligner_index	String specifying bowtie or BWA index. Must be provided when aligner is either "bowtie" or "bwa".
bsgenome	A <a href="#">BSgenome</a> object from which to extract sequences if a <a href="#">GRanges</a> object is provided as input.
n_mismatches	Maximum number of mismatches permitted between guide RNA and genomic DNA.
all_alignments	Should all all possible alignments be returned? FALSE by default.
canonical	TRUE returns only those alignments having canonical PAM sequences; FALSE returns alignments having canonical or noncanonical PAM sequences; NA returns all alignments regardless of their PAM sequence.
standard_chr_only	Should only standard chromosomes be considered? If TRUE, the function will attempt to remove scaffold sequences automatically. FALSE by default.
both_strands	When custom_seq is specified, should both strands be considered? TRUE by default.
anchor	The position within the protospacer as determined by <a href="#">CrisprNuclease</a> to use when annotating with overlapping gene regions.
annotationType	Gene identifier to return when annotating alignments with gene and/or promoter overlaps. Corresponding txObject or tssObject argument must have mcol column name for selected type.
tss_window	Window size of promoters upstream of gene TSS to search for overlap with spacer sequence. Must be a numeric vector of length 2: upstream limit and downstream limit. Default is c(-500, 500), which includes 500bp upstream and downstream of the TSS.
alignmentThresholds	Named numeric vector of the maximum on-target alignments tolerated for <a href="#">addSpacerAlignmentsIterat</a> . Thresholds not provided will take default values.
n_max_alignments	Maximum number of alignments to report by bowtie for each spacer. Effectively set to Inf when allPossible is TRUE.
spacers	Character vector of gRNA spacer sequences. All sequences must be equal in length.
crisprNuclease	A <a href="#">CrisprNuclease</a> object.

### Details

The columns stored in `mcols(guideSet)[["alignments"]]` are:

- spacer Spacer sequence of the query gRNA.
- protospacer Protospacer sequence in the target DNA.
- pam PAM sequence.



- `pam_site` PAM site of the found protospacer.
- `n_mismatches` Integer value specifying the number of nucleotide mismatches between the gRNA spacer sequence and the protospacer sequence found in the genome or custom sequence.
- `canonical` Whether the PAM sequence of the found protospacer sequence is canonical.
- `cut_site` Cut site of the found protospacer.

The following columns are also stored when a `txObject` is provided:

- `cds` Character vector specifying gene names of CDS overlapping the found protospacer sequence.
- `fiveUTRs` Character vector specifying gene names of 5'UTRs overlapping the found protospacer sequence.
- `threeUTRs` Character vector specifying gene names of 3'UTRs overlapping the found protospacer sequence.
- `exons` Character vector specifying gene names of exons overlapping the found protospacer sequence.
- `introns` Character vector specifying gene names of introns overlapping the found protospacer sequence.
- `intergenic` Character vector specifying the nearest gene when the found protospacer sequence is not located in a gene.
- `intergenic_distance` Distance in base pairs from the nearest gene when the found protospacer sequence is not located in a gene.

The following columns are also stored when a `tssObject` is provided:

- `promoters` Character vector specifying gene names of promoters, as defined by `tss_window` relative to the gene TSS, overlapping the found protospacer sequence.

## Value

`getSpacerAlignments` returns a `GRanges` object storing spacer alignment data, including genomic coordinates, spacer and PAM sequences, and position of mismatches relative to `pam_site`.

`addSpacerAlignments` is similar to `getSpacerAlignments`, with the addition of adding the alignment data to a list-column in `mcols(guideSet)` specified by `colname`.

`addSpacerAlignmentsIterative` is similar to `addSpacerAlignments`, except that it avoids finding alignments for spacer sequences that have a large number of on-targets and/or off-targets to speed up the off-target search. The parameters `n0_max`, `n1_max` and `n2_max` specify the maximum number of on-targets (`n0`) and off-targets (`n1` for 1-mismatch off-targets, and `n2` for 2-mismatch off-targets) tolerated before the algorithm stops finding additional off-targets for spacer sequences that exceed those quotas.

## Author(s)

Jean-Philippe Fortin, Luke Hoberecht

**Examples**

```

if (interactive()){
# Creating a bowtie index:
library(Rbowtie)
library(BSgenome.Hsapiens.UCSC.hg38)
fasta <- system.file(package="crisprDesign", "fasta/chr12.fa")
outdir <- tempdir()
Rbowtie::bowtie_build(fasta,
                      outdir=outdir,
                      force=TRUE,
                      prefix="chr12")
bowtieIndex <- file.path(outdir, "chr12")

# Adding spacer alignments with bowtie:
data(guideSetExample, package="crisprDesign")
data(grListExample, package="crisprDesign")
guideSet <- addSpacerAlignments(guideSetExample,
                                aligner="bowtie",
                                aligner_index=bowtieIndex,
                                bsgenome=BSgenome.Hsapiens.UCSC.hg38,
                                n_mismatches=2,
                                txObject=grListExample)
}

```

---

addTssAnnotation      *Add TSS context annotation to a [GuideSet](#) object*

---

**Description**

Add transcription start site (TSS) context annotation to spacer sequences stored in a [GuideSet](#) object.

**Usage**

```

addTssAnnotation(object, ...)

## S4 method for signature 'GuideSet'
addTssAnnotation(
  object,
  tssObject,
  anchor = c("cut_site", "pam_site"),
  tss_window = NULL,
  ignore.strand = TRUE
)

## S4 method for signature 'PairedGuideSet'

```

```

addTssAnnotation(
  object,
  tssObject,
  anchor = c("cut_site", "pam_site"),
  tss_window = NULL,
  ignore.strand = TRUE
)

## S4 method for signature ``NULL``
addTssAnnotation(object)

```

### Arguments

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
tssObject	A <a href="#">GRanges</a> object containing TSS coordinates and annotation.
anchor	A character string specifying which gRNA-specific coordinate to use (cut_site or pam_site) when searching for overlapping TSS regions. "cut_site" by default.
tss_window	A numeric vector of length 2 establishing the window size of the genomic region around the TSS to include as the "TSS region". The values set the upstream and downstream limits, respectively. The default is <code>c(-500, 500)</code> , which includes 500bp upstream (note the negative value) and downstream of the TSS.
ignore.strand	If TRUE (default), includes annotation for gRNAs irrespective of their target strand. Otherwise, only gRNAs targeting the gene strand will be annotated.

### Details

`mcols(guideSet)[["tssAnnotation"]]` includes all columns from `mcols(tssObject)` in addition to the columns described below.

- chr — gRNA chromosome name.
- anchor\_site — Genomic coordinate used to search for overlapping TSS regions.
- strand — Strand the gRNA is located on.
- tss\_id — The ID for the TSS in `tssObject`, if present.
- tss\_strand — Strand the TSS is located on, as provided in `tssObject`
- tss\_pos — Genomic coordinate of the TSS, as provided in `tssObject`.
- dist\_to\_tss — Distance (in nucleotides) between the gRNA `anchor_site` and `tss_pos`. Negative values indicate gRNA targets upstream of the TSS.

### Value

A [GuideSet](#) object with a `tssAnnotation` list column stored in `mcols(guideSet)`. See details section for descriptions of TSS annotation columns.

**Author(s)**

Jean-Philippe Fortin, Luke Hoberecht

**See Also**

[addGeneAnnotation](#) to add gene annotation, and [tssAnnotation](#) to retrieve an existing TSS annotation.

**Examples**

```
data(guideSetExample, package="crisprDesign")
data(tssObjectExample, package="crisprDesign")
guideSet <- addTssAnnotation(guideSetExample,
                           tssObject=tssObjectExample)

# To access TSS annotation:
ann <- tssAnnotation(guideSet)
```

---

addTxTable

*Add a gene-specific transcript table to a [GuideSet](#) object.*

---

**Description**

Add a gene-specific transcript table to a [GuideSet](#) object.

Add a gene-specific transcript table to a [GuideSet](#) object.

**Usage**

```
addTxTable(guideSet, gene_id, txObject, valueColumn = "percentCDS")
```

**Arguments**

guideSet	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
gene_id	String specifying gene ID.
txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained using <a href="#">TxDb2GRangesList</a> to provide a gene model annotation.
valueColumn	String specifying column in <code>geneAnnotation(guideSet)</code> to use as values in the output transcript table.

**Value**

A [GuideSet](#) object with a "txTable" `DataFrame` stored in `mcols(guideSet)`. The entries in the `DataFrame` correspond to the values specified by `valueColumn`. Rows correspond to gRNAs in the `GuideSet`, columns correspond to all transcripts found in `txObject` for gene specified by `gene_id`.

**Author(s)**

Jean-Philippe Fortin

**See Also**

[addGeneAnnotation](#) to add gene annotation.

**Examples**

```
if (interactive()){
  data(guideSetExample, package="crisprDesign")
  data(grListExample, package="crisprDesign")
  guideSet <- addGeneAnnotation(guideSetExample,
                               txObject=grListExample)
  guideSet <- addTxTable(guideSet,
                        gene_id="ENSG00000120645",
                        txObject=grListExample)

  guideSet$txTable
}
```

---

completeSpacers

*Get complete spacer information*

---

**Description**

These functions serve to "fill-in-the-blank" for spacers lacking information.

**Usage**

```
getPAMSequence(chr, pam_site, strand, crisprNuclease = NULL, bsgenome = NULL)
```

```
getSpacerSequence(
  chr,
  pam_site,
  strand,
  crisprNuclease = NULL,
  bsgenome = NULL,
  spacerLen = NULL
)
```

```
getPAMSiteFromStartAndEnd(
  start = NULL,
  end = NULL,
  strand,
  crisprNuclease = NULL,
  spacerLen = NULL
)
```



---

`convertToMinMaxGRanges`

*Convert a GuideSet object into a GRanges containing the range of all targeting gRNAs.*

---

**Description**

Convert a GuideSet object into a GRanges object containing the minimum and maximum coordinates for all targeting gRNAs.

**Usage**

```
convertToMinMaxGRanges(guideSet, anchor = c("cut_site", "pam_site"))
```

**Arguments**

<code>guideSet</code>	A <a href="#">GuideSet</a> object.
<code>anchor</code>	A character string specifying which gRNA-specific coordinate to use ( <code>cut_site</code> or <code>pam_site</code> ) when defining the min and max coordinates of <a href="#">GuideSet</a> object.

**Value**

A GRanges object with start and end coordinates corresponding to the minimum and maximum coordinates of the GuideSet object sites defined by anchor.

**Author(s)**

Jean-Philippe Fortin, Luke Hoberecht

**Examples**

```
data(guideSetExample, package="crisprDesign")
gr <- convertToMinMaxGRanges(guideSetExample)
```

---

`convertToProtospacerGRanges`

*Convert PAM site coordinates to protospacer start and end coordinates*

---

**Description**

Convert PAM site coordinates to protospacer start and end coordinates.

**Usage**

```
convertToProtospacerGRanges(guideSet)
```

**Arguments**

guideSet        A [GuideSet](#) object.

**Value**

A [GuideSet](#) object with start and end coordinates corresponding to the start and end coordinates of the protospacer sequences.

**Author(s)**

Jean-Philippe Fortin

**Examples**

```
data(guideSetExample, package="crisprDesign")
gr <- convertToProtospacerGRanges(guideSetExample)
```

---

crisprNuclease        *An S4 class to store CRISPR gRNA sequences with modular annotations.*

---

**Description**

An S4 class to store CRISPR gRNA sequences with modular annotations.

**Usage**

```
crisprNuclease(object, ...)  
targetOrigin(object, ...)  
customSequences(object, ...)  
bsgenome(object, ...)  
spacers(object, ...)  
protospacers(object, ...)  
pamSites(object, ...)  
snps(object, ...)  
alignments(object, ...)  
onTargets(object, ...)
```



```
offTargets(object, ...)  
geneAnnotation(object, ...)  
tssAnnotation(object, ...)  
enzymeAnnotation(object, ...)  
editedAlleles(object, ...)  
tssAnnotation(object) <- value  
geneAnnotation(object) <- value  
enzymeAnnotation(object) <- value  
snps(object) <- value  
alignments(object) <- value  
addCutSites(object, ...)  
  
GuideSet(  
  ids = NA_character_,  
  protospacers = NA_character_,  
  pams = NULL,  
  seqnames = NA_character_,  
  pam_site = 0L,  
  strand = "*",  
  CrisprNuclease = NULL,  
  targetOrigin = c("bsgenome", "customSequences"),  
  bsgenome = NULL,  
  customSequences = NULL,  
  ...,  
  seqinfo = NULL,  
  seqlengths = NULL  
)  
  
## S4 method for signature 'GuideSet'  
targetOrigin(object)  
  
## S4 method for signature 'GuideSet'  
customSequences(object)  
  
## S4 method for signature 'GuideSet'  
bsgenome(object)
```

```
## S4 method for signature 'GuideSet'
crisprNuclease(object)

## S4 method for signature 'GuideSet'
spacers(object, as.character = FALSE, returnAsRna = FALSE)

## S4 method for signature 'GuideSet'
pams(object, as.character = FALSE, returnAsRna = FALSE)

## S4 method for signature 'GuideSet'
pamSites(object)

## S4 method for signature 'GuideSet'
cutSites(object)

## S4 method for signature 'GuideSet'
addCutSites(object)

## S4 method for signature 'GuideSet'
protospacers(
  object,
  as.character = FALSE,
  include.pam = FALSE,
  returnAsRna = FALSE
)

## S4 method for signature 'GuideSet'
spacerLength(object)

## S4 method for signature 'GuideSet'
prototypeSequence(object)

## S4 method for signature 'GuideSet'
pamLength(object)

## S4 method for signature 'GuideSet'
pamSide(object)

## S4 method for signature 'GuideSet'
snps(object, unlist = TRUE, use.names = TRUE)

## S4 method for signature 'GuideSet'
alignments(object, columnName = "alignments", unlist = TRUE, use.names = TRUE)

## S4 method for signature 'GuideSet'
onTargets(object, columnName = "alignments", unlist = TRUE, use.names = TRUE)

## S4 method for signature 'GuideSet'
```

```
offTargets(  
  object,  
  columnName = "alignments",  
  max_mismatches = Inf,  
  unlist = TRUE,  
  use.names = TRUE  
)  
  
## S4 replacement method for signature 'GuideSet'  
alignments(object) <- value  
  
## S4 replacement method for signature 'GuideSet'  
geneAnnotation(object) <- value  
  
## S4 replacement method for signature 'GuideSet'  
tssAnnotation(object) <- value  
  
## S4 replacement method for signature 'GuideSet'  
enzymeAnnotation(object) <- value  
  
## S4 replacement method for signature 'GuideSet'  
snps(object) <- value  
  
## S4 method for signature 'GuideSet'  
geneAnnotation(  
  object,  
  unlist = TRUE,  
  gene_id = NULL,  
  tx_id = NULL,  
  gene_symbol = NULL,  
  use.names = TRUE  
)  
  
## S4 method for signature 'GuideSet'  
editedAlleles(object)  
  
## S4 method for signature 'GuideSet'  
tssAnnotation(  
  object,  
  unlist = TRUE,  
  gene_id = NULL,  
  gene_symbol = NULL,  
  use.names = TRUE  
)  
  
## S4 method for signature 'GuideSet'  
enzymeAnnotation(object, unlist = TRUE, use.names = TRUE)
```

**Arguments**

object	<a href="#">GuideSet</a> object.
...	Additional arguments for class-specific methods
value	Object to replace with
ids	Character vector of unique gRNA ids. The ids can be anything, as long as they are unique.
protospacers	Character vector of protospacers sequences.
pams	Character vector of PAM sequences.
seqnames	Character vector of chromosome names.
pam_site	Integer vector of PAM site coordinates.
strand	Character vector of gRNA strand. Only accepted values are "+" and "-".
CrisprNuclease	<a href="#">CrisprNuclease</a> object.
targetOrigin	String specifying the origin of the DNA target. Must be either 'bsgenome' or 'customSequences'.
bsgenome	<a href="#">BSgenome</a> object or string specifying BSgenome package name. Must be specified when targetOrigin is set to "bsgenome".
customSequences	<a href="#">DNASTringSet</a> object. Must be specified when targetOrigin is set to "customSequences".
seqinfo	A <a href="#">Seqinfo</a> object containing information about the set of genomic sequences present in the target genome.
seqlengths	NULL, or an integer vector named with levels(seqnames) and containing the lengths (or NA) for each level in levels(seqnames).
as.character	Should sequences be returned as a character vector? FALSE by default, in which case sequences are returned as a <a href="#">DNASTringSet</a> .
returnAsRna	Should the sequences be returned as RNA instead of DNA? FALSE by default.
include.pam	Should PAM sequences be included? FALSE by default.
unlist	Should the annotation be returned as one table instead of a list? TRUE by default.
use.names	Whether to include spacer IDs as (row)names (TRUE), or as a separate column (FALSE).
columnName	Name of the column storing the alignments annotation to be retrieved.
max_mismatches	What should be the maximum number of mismatches considered for off-targets? Inf by default.
gene_id	Character vector of Ensembl gene IDs to subset gene annotation data by. If NULL (default), all genes are considered.
tx_id	Character vector of Ensembl transcript IDs to subset gene annotation data by. If NULL (default), all transcript are considered.
gene_symbol	Character vector of gene symbols to subset gene annotation data by. If NULL (default), all genes are considered.

**Value**

A GuideSet object.

**Functions**

- GuideSet(): Create a [GuideSet](#) object

**Constructors**

Use the constructor `link{GuideSet}` to create a GuideSet object.

**Accessors**

`crisprNuclease`: To get [CrisprNuclease](#) object used to design gRNAs.

`spacers`: To get spacer sequences.

`protospacers`: To get protospacer sequences.

`spacerLength`: To get spacer length.

`pams`: To get PAM sequences.

`pamSites`: To get PAM site coordinates.

`pamLength`: To get PAM length.

`pamSide`: To return the side of the PAM sequence with respect to the protospacer sequence.

`prototypeSequence`: To get a prototype protospacer sequence.

`cutSites`: To get cut sites.

`alignments`: To get genomic alignments annotation.

`onTargets`: To get on-target alignments annotation

`offTargets`: To get off-target alignments annotation

`snps`: To get SNP annotation.

`geneAnnotation`: To get gene annotation.

`tssAnnotation`: To get TSS annotation.

`enzymeAnnotation`: To get restriction enzymes annotation.

`editedAlleles`: To get edited alleles annotation.

**Examples**

```
protospacers <- c("AGGTCGTGTGTGGGGGGGG",
                 "AGGTCGTGTGTGGGGGGGG")
pams <- c("AGG", "CGG")
pam_site=c(10,11)
seqnames="chr7"
data(SpCas9, package="crisprBase")
CrisprNuclease <- SpCas9
strand=c("+", "-")
ids <- paste0("grna_", seq_along(protospacers))
gr <- GuideSet(ids=ids,
```

```

protospacers=protospacers,
pams=pams,
seqnames=seqnames,
CrisprNuclease=CrisprNuclease,
pam_site=pam_site,
strand=strand,
targetOrigin="customSequences",
customSequences=protospacers)

```

---

designCompleteAnnotation

*One-step gRNA design and annotation function*

---

### Description

One-step gRNA design and annotation function to facilitate the design and generation of genome-wide gRNA databases for a combination of parameters such as nuclease, organism, and CRISPR modality.

### Usage

```

designCompleteAnnotation(
  queryValue = NULL,
  queryColumn = "gene_id",
  featureType = "cds",
  modality = c("CRISPRko", "CRISPRa", "CRISPRi", "CRISPRkd"),
  bsgenome = NULL,
  bowtie_index = NULL,
  vcf = NULL,
  crisprNuclease = NULL,
  tssObject = NULL,
  txObject = NULL,
  grRepeats = NULL,
  scoring_methods = NULL,
  tss_window = NULL,
  n_mismatches = 3,
  max_mm = 2,
  canonical_ontarget = TRUE,
  canonical_offtarget = FALSE,
  all_alignments = TRUE,
  fastaFile = NULL,
  chromatinFiles = NULL,
  geneCol = "gene_symbol",
  conservationFile = NULL,
  nucExtension = 9,
  binaries = NULL,
  canonicalIsoforms = NULL,

```

```

    pfamTable = NULL,
    verbose = TRUE
)

```

## Arguments

queryValue	Vector specifying the value(s) to search for in txObject[[featureType]][[queryColumn]].
queryColumn	Character string specifying the column in txObject[[featureType]] to search for queryValue(s).
featureType	For CRISPRko, string specifying the type of genomic feature to use to design gRNAs. Must be of the following: "transcripts", "exons", "cds", "fiveUTRs", "threeUTRs" or "introns". The default is "cds".
modality	String specifying the CRISPR modality. Must be one of the following: "CRISPRko", "CRISPRa", "CRISPRi" or "CRISPRkd". CRISPRkd is reserved for DNA-targeting nucleases only such as CasRx.
bsgenome	A <a href="#">BSgenome</a> object from which to extract sequences if a <a href="#">GRanges</a> object is provided as input.
bowtie_index	String specifying path to a bowtie index.
vcf	Either a character string specifying a path to a VCF file or connection, or a <a href="#">VCF</a> object.
crisprNuclease	A <a href="#">CrisprNuclease</a> object.
tssObject	A <a href="#">GRanges</a> object specifying TSS coordinates.
txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained using <a href="#">TxDb2GRangesList</a> for annotating on-target and off-target alignments using gene annotation.
grRepeats	A <a href="#">GRanges</a> object containing repeat elements regions.
scoring_methods	Character vector to specify which on-target scoring methods should be calculated. See <a href="#">crisprScore</a> package to obtain available methods.
tss_window	Vector of length 2 specifying the start and coordinates of the CRISPRa/CRISPRi target region with respect to the TSS position.
n_mismatches	Maximum number of mismatches permitted between guide RNA and genomic DNA.
max_mm	The maximum number of mismatches between a spacer and an off-target to be accepted when calculating aggregate off-target scores. 2 by default.
canonical_ontarget	Should only canonical PAM sequences be searched for designing gRNAs? TRUE by default.
canonical_offtarget	Should only canonical PAM sequences be searched during the off-target search? TRUE by default.
all_alignments	Should all all possible alignments be returned? TRUE by default.
fastaFile	String specifying fasta file of the hg38 genome. Only used for CRISPRa/i modality with hg38 genome and SpCas9 nuclease. This is needed to generate the CRISPRai scores. See the function <a href="#">addCrispraiScores</a> for more details.

chromatinFiles	Named character vector of length 3 specifying BigWig files containing chromatin accessibility data. Only used for CRISPRa/i modality with hg38 genome and SpCas9 nuclease. This is needed to generate the CRISPRai scores. See the function <code>addCrispraiScores</code> for more details.
geneCol	String specifying the column in the <code>tssObject</code> to be used to specify the gene name for the <code>addCrispraiScores</code> function. "gene_symbol" by default.
conservationFile	String specifying the BigWig file containing conservation scores.
nucExtension	Number of nucleotides to include on each side of the cut site to calculate the conservation score. 9 by default. The region will have $(2 * \text{nucExtension} + 1)$ nucleotides in total.
binaries	Named list of paths for binaries needed for CasRx-RF. Names of the list must be "RNAfold", "RNAhybrid", and "RNAplfold". Each list element is a string specifying the path of the binary. If NULL (default), binaries must be available on the PATH.
canonicalIsoforms	Optional data.frame with 2 columns detailing Ensembl canonical isoforms. First column must be named "tx_id", and second column must be named "gene_id", corresponding to Ensembl transcript and gene ids, respectively.
pfamTable	A <a href="#">DataFrame</a> obtained using <a href="#">preparePfamTable</a> .
verbose	Should messages be printed?

**Value**

A `GuideSet` object.

**Author(s)**

Jean-Philippe Fortin

---

designOpsLibrary

*Design gRNA library for optical pooled screening*

---

**Description**

Design gRNA library for optical pooled screening

**Usage**

```
designOpsLibrary(
  df,
  n_guides = 4,
  gene_field = "gene",
  min_dist_edit = 2,
  dist_method = c("hamming", "levenshtein"),
  splitByChunks = FALSE
)
```



**Arguments**

df	data.frame containing information about candidate gRNAs from which to build the OPS library. See details.
n_guides	Integer specifying how many gRNAs per gene should be selected. 4 by default.
gene_field	String specifying the column in df specifying gene names.
min_dist_edit	Integer specifying the minimum distance edit required for barcodes to be considered dissimilar. Barcodes that have edit distances less than the min_dist_edit will not be included in the library. 2 by default.
dist_method	String specifying distance method. Must be either "hamming" (default) or "levenshtein".
splitByChunks	Should distances be calculated in a chunk-wise manner? FALSE by default. Highly recommended when the set of query barcodes is large to reduce memory footprint.

**Value**

A subset of the df containing the gRNAs selected for the OPS library.

**Author(s)**

Jean-Philippe Fortin

**Examples**

```
data(guideSetExample, package="crisprDesign")
guideSet <- unique(guideSetExample)
guideSet <- addOpsBarcodes(guideSet)
guideSet <- guideSet[1:200]

df <- data.frame(ID=names(guideSet),
                 spacer=spacers(guideSet, as.character=TRUE),
                 opsBarcode=as.character(guideSet$opsBarcode))

# Creating mock gene:
df$gene <- rep(paste0("gene", 1:10), each=20)
df$rank <- rep(1:20, 10)
opsLib <- designOpsLibrary(df)
```

---

findSpacerPairs

*Find pairs of CRISPR gRNA spacers from a pair of genomic regions.*

---

**Description**

Returns all possible, valid gRNA sequences for a given CRISPR nuclease from either a [GRanges](#) object or a set of sequence(s) contained in either a [DNAStrngSet](#), [DNAStrng](#) or character vector of genomic sequences.

**Usage**

```
findSpacerPairs(
  x1,
  x2,
  sortWithinPair = TRUE,
  pamOrientation = c("all", "out", "in"),
  minCutLength = NULL,
  maxCutLength = NULL,
  crisprNuclease = NULL,
  bsgenome = NULL,
  canonical = TRUE,
  both_strands = TRUE,
  spacer_len = NULL,
  strict_overlap = TRUE,
  remove_ambiguities = TRUE
)
```

**Arguments**

x1	Either a <a href="#">GRanges</a> , a <a href="#">DNASTringSet</a> , or a <a href="#">DNASTring</a> object, or a character vector of genomic sequences. This specifies the sequence space from which gRNAs in position 1 of the pairs will be designed. Alternatively, a <a href="#">GuideSet</a> object can be provided.
x2	Either a <a href="#">GRanges</a> , a <a href="#">DNASTringSet</a> , or a <a href="#">DNASTring</a> object, or a character vector of genomic sequences. This specifies the sequence space from which gRNAs in position 2 of the pairs will be designed. Alternatively, a <a href="#">GuideSet</a> object can be provided.
sortWithinPair	Should gRNAs be sorted by chr and position within a pair? TRUE by default.
pamOrientation	String specifying a constraint on the PAM orientation of the pairs. Should be either "all" (default), "out" (for the so-called PAM-out orientation) or "in" (for PAM-in orientation).
minCutLength	Integer specifying the minimum cut length allowed (distance between the two cuts) induced by the gRNA pair. If NULL (default), the argument is ignored. Note that this parameter is only applicable for pairs of gRNAs targeting the same chromosome.
maxCutLength	Integer specifying the maximum cut length allowed (distance between the two cuts) induced by the gRNA pair. If NULL (default), the argument is ignored. Note that this parameter is only applicable for pairs of gRNAs targeting the same chromosome.
crisprNuclease	A <a href="#">CrisprNuclease</a> object.
bsgenome	A <a href="#">BSgenome</a> object from which to extract sequences if x is a <a href="#">GRanges</a> object.
canonical	Whether to return only guide sequences having canonical PAM sequences. If TRUE (default), only PAM sequences with the highest weights stored in the <a href="#">crisprNuclease</a> object will be considered.
both_strands	Whether to consider both strands in search for protospacer sequences. TRUE by default.

spacer_len	Length of spacers to return, if different from the default length specified by <code>crisprNuclease</code> .
strict_overlap	Whether to only include gRNAs that cut in the input range, as given by <code>cut_site</code> (TRUE) or to include all gRNAs that share any overlap with the input range (FALSE). TRUE by default. Ignored when <code>x</code> is not a <a href="#">GRanges</a> object.
remove_ambiguities	Whether to remove spacer sequences that contain ambiguous nucleotides (not explicitly A, C, G, or T). TRUE by default.

### Details

This function returns a [PairedGuideSet](#) object that stores gRNA pairs targeting the two genomic regions provided as input. The gRNAs in position 1 target the first genomic region, and the gRNAs in position 2 target the second genomic region.

This function can be used for the following scenarios:

1. Designing pairs of gRNAs targeting different genes, for instance for dual-promoter Cas9 systems, or polycistronic Cas12a constructs. This can also be used to target a given gene with multiple gRNAs for improved efficacy (for instance CRISPRa and CRISPRi)
2. Designing pairs of gRNAs for double nicking systems such as Cas9 D10A.

See vignette for more examples.

### Value

A [PairedGuideSet](#) object.

### Author(s)

Jean-Philippe Fortin

### See Also

[findSpacers](#) to find unpaired spacer sequences, and the [PairedGuideSet](#) object documentation to understand the output of `findSpacerPairs`.

### Examples

```
library(GenomicRanges)
library(BSgenome.Hsapiens.UCSC.hg38)
library(crisprBase)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38

# Region 1:
gr1 <- GRanges(c("chr12"),
               IRanges(start=22224014, end=22225007))

# Region 2:
gr2 <- GRanges(c("chr13"),
               IRanges(start=23224014, end=23225007))
```

```

# Pairs targeting the same region:
pairs <- findSpacerPairs(gr1, gr1, bsgenome=bsgenome)

# Pairs targeting two regions:
# The gRNA in position targets gr1
# and the gRNA in position 2 targets gr2
pairs <- findSpacerPairs(gr1, gr2, bsgenome=bsgenome)

```

---

findSpacers

*Find CRISPR gRNA spacer sequences from a set of DNA sequences.*


---

### Description

Returns all possible, valid gRNA sequences for a given CRISPR nuclease from either a [GRanges](#) object or a set of sequence(s) contained in either a [DNAStringSet](#), [DNAString](#) or character vector of genomic sequences.

### Usage

```

findSpacers(
  x,
  crisprNuclease = NULL,
  bsgenome = NULL,
  canonical = TRUE,
  both_strands = TRUE,
  spacer_len = NULL,
  strict_overlap = TRUE,
  remove_ambiguities = TRUE,
  remove_duplicates = TRUE
)

```

### Arguments

x	Either a <a href="#">GRanges</a> , a <a href="#">DNAStringSet</a> , or a <a href="#">DNAString</a> object, or a character vector of genomic sequences. See details.
crisprNuclease	A <a href="#">CrisprNuclease</a> object.
bsgenome	A <a href="#">BSgenome</a> object from which to extract sequences if x is a <a href="#">GRanges</a> object.
canonical	Whether to return only guide sequences having canonical PAM sequences. If TRUE (default), only PAM sequences with the highest weights stored in the <a href="#">crisprNuclease</a> object will be considered.
both_strands	Whether to consider both strands in search for protospacer sequences. TRUE by default.
spacer_len	Length of spacers to return, if different from the default length specified by <a href="#">crisprNuclease</a> .

- `strict_overlap` Whether to only include gRNAs that cut in the input range, as given by `cut_site` (TRUE) or to include all gRNAs that share any overlap with the input range (FALSE). TRUE by default. Ignored when `x` is not a [GRanges](#) object.
- `remove_ambiguities` Whether to remove spacer sequences that contain ambiguous nucleotides (not explicitly A, C, G, or T). TRUE by default.
- `remove_duplicates` Whether to remove duplicated protospacer sequences originating from overlapping genomic ranges. TRUE by default.

### Details

If `x` is a [GRanges](#) object then a [BSgenome](#) must be supplied to `bsgenome`, from which the genomic sequence is obtained, unless the `bsgenome` can be inferred from `genome(x)`, for example, "hg38". Otherwise, all supplied sequences are treated as the "+" strands of chromosomes in a "custom" genome.

Ranges or sequences in `x` may contain names where permitted. These names are stored in `region` in the `mcols` of the output, and as `seqnames` of the output if `x` is not a [GRanges](#) object. If not NULL, `names(x)` must be unique, otherwise ranges or sequences are enumerated with the "region\_" prefix.

When `x` is a [GRanges](#), the `*` strand is interpreted as both strands. Consequently, the `both_strands` argument has no effect on such ranges.

### Value

A [GuideSet](#) object.

### Author(s)

Jean-Philippe Fortin, Luke Hoberecht

### Examples

```
# Using custom sequence as input:
my_seq <- c(my_seq="CCAANAGTGAAACACGTCTCTATAAAGAATACAAAAAATTAGCCGGGTGTTA")
guides <- findSpacers(my_seq)

# Exon-intro region of human KRAS specified
# using a GRanges object:
library(GenomicRanges)
library(BSgenome.Hsapiens.UCSC.hg38)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38

gr_input <- GRanges(c("chr12"),
                    IRanges(start=25224014, end=25227007))
guideSet <- findSpacers(gr_input, bsgenome=bsgenome)

# Designing guides for enAsCas12a nuclease:
data(enAsCas12a, package="crisprBase")
guideSet <- findSpacers(gr_input,
```

```
canonical=FALSE,
bsgenome=bsgenome,
crisprNuclease=enAsCas12a)
```

---

flattenGuideSet	<i>Create a list of annotation tables from a GuideSet object</i>
-----------------	--

---

### Description

Create a list of annotation tables from a GuideSet object

### Usage

```
flattenGuideSet(guideSet, useSpacerCoordinates = TRUE, primaryOnly = FALSE)
```

### Arguments

guideSet	A GuideSet object
useSpacerCoordinates	Should the spacer coordinates be used as start and end coordinates? TRUE by default. If FALSE, the PAM site coordinate is used for both start and end.
primaryOnly	Should only the primary table (on-targets) be returned? FALSE by default.

### Value

A simple list of tables containing annotations derived from a GuideSet object. The first table ("primary") is always available, while the other tables will be only available when the annotations were added to the GuideSet object.

- `primary` Primary table containing genomic coordinates and sequence information of the gRNA sequences. Also contains on-target and off-target scores when available.
- `alignments` Table of on- and off-target alignments.
- `geneAnnotation` Gene context annotation table.
- `tssAnnotation` TSS context annotation table.
- `enzymeAnnotation` Boolean table indicating whether or not recognition motifs of restriction enzymes are found.
- `snps` SNP annotation table (human only).

### Author(s)

Jean-Philippe Fortin

---

`getBarcodeDistanceMatrix`*Get distance between query and target sets of barcodes*

---

**Description**

Get distance between query and target sets of barcodes

**Usage**

```
getBarcodeDistanceMatrix(  
  queryBarcodes,  
  targetBarcodes = NULL,  
  binarize = TRUE,  
  min_dist_edit = NULL,  
  dist_method = c("hamming", "levenshtein"),  
  ignore_diagonal = TRUE,  
  splitByChunks = FALSE,  
  n_chunks = NULL  
)
```

**Arguments**

<code>queryBarcodes</code>	Character vector of DNA sequences or <code>DNAStrngSet</code> .
<code>targetBarcodes</code>	Optional character vector of DNA sequences or <code>DNAStrngSet</code> . If <code>NULL</code> , distances will be calculated between barcodes provided in <code>queryBarcodes</code> .
<code>binarize</code>	Should the distance matrix be made binary? <code>TRUE</code> by default. See details section.
<code>min_dist_edit</code>	Integer specifying the minimum distance edit required for barcodes to be considered dissimilar when <code>binarize=TRUE</code> , ignored otherwise.
<code>dist_method</code>	String specifying distance method. Must be either "hamming" (default) or "levenshtein".
<code>ignore_diagonal</code>	When <code>targetBarcodes=NULL</code> , should the diagonal distances be set to 0 to ignore self distances? <code>TRUE</code> by default.
<code>splitByChunks</code>	Should distances be calculated in a chunk-wise manner? <code>FALSE</code> by default. Highly recommended when the set of query barcodes is large to reduce memory footprint.
<code>n_chunks</code>	Integer specifying the number of chunks to be used when <code>splitByChunks=TRUE</code> . If <code>NULL</code> (default), number of chunks will be chosen automatically.

**Value**

A sparse matrix of class `dgMatrix` or `dsMatrix` in which rows correspond to `queryBarcodes` and columns correspond to `targetBarcodes`. If `binarize=TRUE`, a value of 0 indicates that two barcodes have a distance greater of equal to `min_dist_edit`, otherwise the value is 1. If `binarize=FALSE`, values represent the actual calculated distances between barcodes.

**Author(s)**

Jean-Philippe Fortin

**Examples**

```
data(guideSetExample, package="crisprDesign")
guideSetExample <- addOpsBarcodes(guideSetExample)
barcodes <- as.character(guideSetExample$opsBarcode)
dist <- getBarcodeDistanceMatrix(barcodes, min_dist_edit=2)
```

---

getConsensusIsoform     *Get the genomic ranges of a consensus isoform*

---

**Description**

Get the genomic ranges of a consensus isoform. The consensus isoform is taken as the union of exons across all isoforms where overlapping exons are merged to produce a simplified set through the reduce method of the GenomicRanges package.

**Usage**

```
getConsensusIsoform(gene_id, txObject)
```

**Arguments**

gene_id	String specifying Ensembl ID for the gene of interest. E.g. "ENSG00000049618". ID must be present in txObject\$exons\$gene_id.
txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained using <a href="#">TxDb2GRangesList</a> to provide a gene model annotation.

**Value**

A GRanges object.

**Author(s)**

Jean-Philippe Fortin

**Examples**

```
data(grListExample)
gene_id <- "ENSG00000120645"
gr <- getConsensusIsoform(gene_id, grListExample)
```



---

getMrnaSequences	<i>Retrieve mRNA sequences</i>
------------------	--------------------------------

---

### Description

A function for retrieving mRNA sequences of select transcripts.

### Usage

```
getMrnaSequences(txids, txObject, bsgenome)
```

### Arguments

txids	A character vector of Ensembl transcript IDs. IDs not present in txObject will be silently ignored.
txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained from <a href="#">TxDb2GRangesList</a> . Defines genomic ranges for txids.
bsgenome	A <a href="#">BSgenome</a> object from which to extract mRNA sequences.

### Value

A [DNAStringSet](#) object of mRNA sequences. Note that sequences are returned as DNA rather than RNA.

### Author(s)

Jean-Philippe Fortin

### Examples

```
library(BSgenome.Hsapiens.UCSC.hg38)
data(grListExample)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38
txids <- c("ENST00000538872", "ENST00000382841")
out <- getMrnaSequences(txids, grListExample, bsgenome)
```

---

getPreMrnaSequences    *Retrieve pre-mRNA sequences*

---

### Description

A function for retrieving pre-mRNA sequences of select transcripts.

### Usage

```
getPreMrnaSequences(txids, txObject, bsgenome)
```

### Arguments

txids	A character vector of Ensembl transcript IDs. IDs not present in txObject will be silently ignored.
txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained from <a href="#">TxDb2GRangesList</a> . Defines genomic ranges for txids.
bsgenome	A <a href="#">BSgenome</a> object from which to extract pre-mRNA sequences.

### Value

A [DNAStringSet](#) object of mRNA sequences. Note that sequences are returned as DNA rather than RNA.

### Author(s)

Jean-Philippe Fortin

### Examples

```
library(BSgenome.Hsapiens.UCSC.hg38)
data(grListExample)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38
txids <- c("ENST00000538872", "ENST00000382841")
out <- getPreMrnaSequences(txids, grListExample, bsgenome)
```

---

`getTssObjectFromTxObject`*Extract TSS coordinates from a gene model object*

---

**Description**

Extract TSS coordinates from a gene model object.

**Usage**

```
getTssObjectFromTxObject(txObject)
```

**Arguments**

`txObject` A [TxDb](#) object or a [GRangesList](#) object obtained using [TxDb2GRangesList](#) for annotating on-target and off-target alignments using gene annotation.

**Value**

A [GRanges](#) object containing TSS coordinates

**Author(s)**

Jean-Philippe Fortin

**Examples**

```
data(grListExample, package="crisprDesign")
tss <- getTssObjectFromTxObject(grListExample)
```

---

`getTxDb`*getTxDb*

---

**Description**

Convenience function for constructing a [TxDb](#) object.

**Usage**

```
getTxDb(file = NA, organism, release = NA, tx_attrib = "gencode_basic", ...)
```

**Arguments**

file	File argument for <code>makeTxDbFromGFF</code> (see help page for <code>makeTxDbFromGFF</code> ). If NA (default), function will return a <code>TxDb</code> object from Ensembl using <code>makeTxDbFromEnsembl</code> .
organism	String specifying genus and species name (e.g. "Homo sapiens" for human). Required if file is not provided. If file is provided, this value can be set to NA to have organism information as unspecified.
release	Ensembl release version; passed to <code>makeTxDbFromEnsembl</code> when file is not specified. See help page for <code>makeTxDbFromEnsembl</code> .
tx_attr	Argument passed to <code>makeTxDbFromEnsembl</code> when file is not specified. See help page for <code>makeTxDbFromEnsembl</code> .
...	Additional arguments passed to either <code>makeTxDbFromGFF</code> (if file is specified) or <code>makeTxDbFromEnsembl</code> if file is NA.

**Value**

A `TxDb` object.

**Author(s)**

Jean-Philippe Fortin, Luke Hoberecht

**Examples**

```
if (interactive()){
  # To obtain a TxDb for Homo sapiens from Ensembl:
  txdb <- getTxDb()

  # To obtain a TxDb from a GFF file:
  file='https://www.mirbase.org/ftp/CURRENT/genomes/hsa.gff3'
  txdb <- getTxDb(file=file)
}
```

---

`getTxInfoDataFrame`      *To obtain a DataFrame of transcript-specific CDS and mRNA coordinates*

---

**Description**

To obtain a `DataFrame` of transcript-specific CDS and mRNA coordinates.

**Usage**

```
getTxInfoDataFrame(
  tx_id,
  txObject,
  bsgenome,
  extend = 30,
  checkCdsLength = TRUE
)
```

**Arguments**

tx_id	String specifying ENSEMBL Transcript id.
txObject	A <code>TxDB</code> object or a <code>GRangesList</code> object obtained using <code>TxDB2GRangesList</code> .
bsgenome	<code>BSgenome</code> object from which to extract sequences if a <code>GRanges</code> object is provided as input.
extend	Integer value specifying how many nucleotides in intron regions should be included.
checkCdsLength	Should the CDS nucleotide length be a multiple of 3? TRUE by default.

**Value**

A `DataFrame` containing nucleotide and amino acid information. The columns are:

- chr Character specifying chromosome.
- pos Integer value specifying coordinate in reference genome.
- strand Character specifying strand of transcript.
- nuc Character specifying nucleotide on the strand specified by strand.
- aa Character specifying amino acid.
- aa\_number Integer specifying amino acid number from 5' end.
- exon Integer specifying exon number.
- pos\_plot Integer specifying plot coordinate. Useful for plotting.
- pos\_mrna Integer specifying relative mRNA coordinate from the start of the mRNA.
- pos\_cds Integer specifying relative CDS coordinate from the start of the CDS.
- region Character specifying gene region: 3UTR, 5UTR, CDS, Intron, Upstream (promoter) or downstream.

**Author(s)**

Jean-Philippe Fortin

**Examples**

```
library(BSgenome.Hsapiens.UCSC.hg38)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38
data("grListExample")
tx_id <- "ENST00000538872"
df <- getTxInfoDataFrame(tx_id=tx_id,
  txObject=grListExample,
  bsgenome=bsgenome)
```

---

`grListExample`*Example of a [TxDb](#) object converted to a [GRangesList](#)*

---

**Description**

Example of a [TxDb](#) object converted to a [GRangesList](#) object for human gene IQSEC3 (ENSG00000120645).

**Usage**

```
data(grListExample, package="crisprDesign")
```

**Format**

Named [GRangesList](#) with 7 elements: transcripts, exons, cds, fiveUTRs, threeUTRs, introns and tss.

**Details**

The full human transcriptome [TxDb](#) object was obtained from the Ensembl 104 release using the [getTxDb](#) function and converted to a [GRangesList](#) object using the [TxDb2GRangesList](#) function and subsequently subsetting to only contain the IQSEC3 gene (ENSG00000120645) located at the start of chr12 in the human genome (hg38 build).

---

`grRepeatsExample`*Example of a [GRanges](#) object containing repeat elements*

---

**Description**

Example of a [GRanges](#) object containing genomic coordinates of repeat elements found in the neighborhood of human gene IQSEC3 (ENSG00000120645).

**Usage**

```
data(grRepeatsExample, package="crisprDesign")
```

**Format**

A [GRanges](#) object.

---

GuideSet2DataFrames    *Create a list of annotation tables from a GuideSet object*

---

## Description

Create a list of annotation tables from a GuideSet object

## Usage

```
GuideSet2DataFrames(guideSet, useSpacerCoordinates = TRUE, primaryOnly = FALSE)
```

## Arguments

<code>guideSet</code>	A GuideSet object
<code>useSpacerCoordinates</code>	Should the spacer coordinates be used as start and end coordinates? TRUE by default. If FALSE, the PAM site coordinate is used for both start and end.
<code>primaryOnly</code>	Should only the primary table (on-targets) be returned? FALSE by default.

## Value

A simple list of tables containing annotations derived from a GuideSet object. The first table ("primary") is always available, while the other tables will be only available when the annotations were added to the GuideSet object.

- `primary` Primary table containing genomic coordinates and sequence information of the gRNA sequences. Also contains on-target and off-target scores when available.
- `alignments` Table of on- and off-target alignments.
- `geneAnnotation` Gene context annotation table.
- `tssAnnotation` TSS context annotation table.
- `enzymeAnnotation` Boolean table indicating whether or not recognition motifs of restriction enzymes are found.
- `snps` SNP annotation table (human only).

## Author(s)

Jean-Philippe Fortin

## Examples

```
data(guideSetExampleFullAnnotation)
tables <- GuideSet2DataFrames(guideSetExampleFullAnnotation)
```

---

guideSetExample	<i>Example of a <a href="#">GuideSet</a> object storing gRNA sequences targeting the CDS of IQSEC3</i>
-----------------	--

---

**Description**

Example of a [GuideSet](#) object storing gRNA sequences targeting the coding sequence of human gene IQSEC3 (ENSG00000120645) for SpCas9 nuclease.

**Usage**

```
data(guideSetExample, package="crisprDesign")
```

**Format**

A [GuideSet](#) object.

**Details**

The object was obtained by calling [findSpacers](#) on the CDS region of human gene IQSEC3. See code in `inst/scripts/generateGuideSet.R`.

---

guideSetExampleFullAnnotation	<i>Example of a fully-annotated <a href="#">GuideSet</a> object storing gRNA sequences targeting the CDS of IQSEC3</i>
-------------------------------	--

---

**Description**

Example of a fully-annotated [GuideSet](#) object storing gRNA sequences targeting the coding sequence of human gene IQSEC3 (ENSG00000120645) for SpCas9 nuclease.

**Usage**

```
data(guideSetExampleFullAnnotation, package="crisprDesign")
```

**Format**

A [GuideSet](#) object.

**Details**

The object was obtained by applying all available `add*` annotation functions in `crisprDesign` (e.g. `addSequenceFeatures`) to a randomly selected 20-guide subset of `guideSetExample`. See code in `inst/scripts/generateGuideSetFullAnnotation.R`.



---

guideSetExampleWithAlignments

*Example of a [GuideSet](#) object storing gRNA sequences targeting the CDS of IQSEC3 with off-target alignments.*

---

### Description

Example of a [GuideSet](#) object storing gRNA sequences targeting the coding sequence of human gene IQSEC3 (ENSG00000120645) for SpCas9 nuclease with off-target alignments.

### Usage

```
data(guideSetExampleWithAlignments, package="crisprDesign")
```

### Format

A [GuideSet](#) object.

### Details

The object was obtained by adding off-target alignments to a randomly selected 20-guide subset of `guideSetExample`. See code in `inst/scripts/generateGuideSetFullAnnotation.R`.

---

pamOrientation      *An S4 class to store pairs of CRISPR gRNA sequences.*

---

### Description

An S4 class to store pairs of CRISPR gRNA sequences.

### Usage

```
pamOrientation(object, ...)
```

```
pamDistance(object, ...)
```

```
spacerDistance(object, ...)
```

```
cutLength(object, ...)
```

```
PairedGuideSet(GuideSet1 = NULL, GuideSet2 = NULL)
```

```
## S4 method for signature 'PairedGuideSet'  
pamOrientation(object)
```

```

## S4 method for signature 'PairedGuideSet'
pamDistance(object)

## S4 method for signature 'PairedGuideSet'
spacerDistance(object)

## S4 method for signature 'PairedGuideSet'
cutLength(object)

## S4 method for signature 'PairedGuideSet'
crisprNuclease(object, index = NULL)

## S4 method for signature 'PairedGuideSet'
spacers(object, as.character = FALSE, returnAsRna = FALSE, index = NULL)

## S4 method for signature 'PairedGuideSet'
pams(object, as.character = FALSE, returnAsRna = FALSE, index = NULL)

## S4 method for signature 'PairedGuideSet'
pamSites(object, index = NULL)

## S4 method for signature 'PairedGuideSet'
cutSites(object, index = NULL)

## S4 method for signature 'PairedGuideSet'
protospacers(
  object,
  as.character = FALSE,
  include.pam = FALSE,
  returnAsRna = FALSE,
  index = NULL
)

## S4 method for signature 'PairedGuideSet'
spacerLength(object, index = NULL)

## S4 method for signature 'PairedGuideSet'
pamLength(object, index = NULL)

## S4 method for signature 'PairedGuideSet'
pamSide(object, index = NULL)

```

### Arguments

<code>object</code>	A <a href="#">PairedGuideSet</a> object.
<code>...</code>	Additional arguments for class-specific methods
<code>GuideSet1</code>	A <a href="#">GuideSet</a> object containing gRNAs at the first position of the pairs.
<code>GuideSet2</code>	A <a href="#">GuideSet</a> object containing gRNAs at the second position of the pairs.

index	Integer value indicating gRNA position. Must be either 1, 2, or NULL (default). If NULL, both positions are returned.
as.character	Should sequences be returned as a character vector? FALSE by default, in which case sequences are returned as a <a href="#">DNAStrngSet</a> .
returnAsRna	Should the sequences be returned as RNA instead of DNA? FALSE by default.
include.pam	Should PAM sequences be included? FALSE by default.

**Value**

A PairedGuideSet object.

**Functions**

- PairedGuideSet(): Create a [PairedGuideSet](#) object

**Constructors**

Use the constructor `link{PairedGuideSet}` to create a PairedGuideSet object.

**Examples**

```
library(crisprDesign)
data(guideSetExample, package="crisprDesign")
gs <- guideSetExample
gs <- gs[order(BiocGenerics::start(gs))]
gs1 <- gs[1:10]
gs2 <- gs[1:10+10]
pgs <- PairedGuideSet(gs1, gs2)
```

---

```
preparePfamTable
```

*Obtain Pfam domains from biomaRt*

---

**Description**

Obtain Pfam domains from biomaRt for all transcripts found in a gene model object.

**Usage**

```
preparePfamTable(txObject, mart_dataset)
```

**Arguments**

txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained using <a href="#">TxDb2GRangesList</a> to provide a gene model annotation.
mart_dataset	String specifying dataset to be used by <b>biomaRt</b> for Pfam domains annotation . E.g. "hsapiens_gene_ensembl".

**Value**

A [DataFrame](#) object with the following columns:

- `ensembl_transcript_id` Ensembl transcript ID.
- `pfam` Pfam domain name.
- `pfam_start` Start amino acid coordinate of the Pfam domain.
- `pfam_end` End amino acid coordinate of the Pfam domain.

**Author(s)**

Jean-Philippe Fortin, Luke Hoberecht

**Examples**

```
data(grListExample, package="crisprDesign")

if (interactive()){
  pfamTable <- preparePfamTable(grListExample,
                                mart_dataset="hsapiens_gene_ensembl")
}
```

---

queryTss

*Convenience function to search for TSS coordinates.*

---

**Description**

Convenience function to search for TSS coordinates.

**Usage**

```
queryTss(tssObject, queryColumn, queryValue, tss_window = NULL)
```

**Arguments**

<code>tssObject</code>	A <a href="#">GRanges</a> containing genomic positions of transcription starting sites (TSSs).
<code>queryColumn</code>	String specifying which column of <code>mcols(tssObject)</code> should be searched for.
<code>queryValue</code>	Character vector specifying the values to search for in <code>tssObject[[queryColumn]]</code> .
<code>tss_window</code>	Numeric vector of length 2 establishing the genomic region to return. The value pair sets the 5 prime and 3 prime limits, respectively, of the genomic region with respect to the TSS. Use negative value(s) to set limit(s) upstream of the TSS. Default is <code>c(-500, 500)</code> , which includes 500bp upstream and downstream of the TSS.

**Value**

A [GRanges](#) object. Searches yielding no results will return an empty [GRanges](#) object.

**Author(s)**

Luke Hoberecht, Jean-Philippe Fortin

**See Also**

[queryTxObject](#) for querying gene annotations.

**Examples**

```
data(tssObjectExample, package="crisprDesign")
queryTss(tssObjectExample,
         queryColumn="gene_symbol",
         queryValue="IQSEC3")
```

---

queryTxObject

*Convenience function to search for gene coordinates.*

---

**Description**

Convenience function to search for gene coordinates.

**Usage**

```
queryTxObject(
  txObject,
  featureType = c("transcripts", "exons", "cds", "fiveUTRs", "threeUTRs", "introns"),
  queryColumn,
  queryValue
)
```

**Arguments**

txObject	A <a href="#">TxDb</a> object or a <a href="#">GRangesList</a> object obtained using <a href="#">TxDb2GRangesList</a> .
featureType	The genomic feature in txObject to base your query on. Must be one of the following: "transcripts", "exons", "cds", "fiveUTRs", "threeUTRs" or "introns".
queryColumn	Character string specifying the column in txObject[[featureType]] to search for queryValue(s).
queryValue	Vector specifying the value(s) to search for in txObject[[featureType]][[queryColumn]].

**Value**

A [GRanges](#) object. Searches yielding no results will return an empty [GRanges](#) object.

**Author(s)**

Luke Hoberecht, Jean-Philippe Fortin

**See Also**

[queryTss](#) for querying TSS annotations.

**Examples**

```
data(grListExample, package="crisprDesign")
queryTxObject(grListExample,
              featureType="cds",
              queryColumn="gene_symbol",
              queryValue="IQSEC3")
```

---

rankSpacers	<i>Recommended gRNA ranking</i>
-------------	---------------------------------

---

**Description**

Function for ranking spacers using recommended crisprDesign criteria. CRISPRko, CRISPRa and CRISPRi modalities are supported.

**Usage**

```
rankSpacers(
  guideSet,
  tx_id = NULL,
  commonExon = FALSE,
  modality = c("CRISPRko", "CRISPRa", "CRISPRi"),
  useDistanceToTss = TRUE
)
```

**Arguments**

guideSet	A <a href="#">GuideSet</a> object.
tx_id	Optional string specifying transcript ID to use isoform-specific information for gRNA ranking.
commonExon	Should gRNAs targeting common exons by prioritized? FALSE by default. If TRUE, tx_id must be provided.
modality	String specifying the CRISPR modality. Should be one of the following: "CRISPRko", "CRISPRa", or "CRISPRi".
useDistanceToTss	Should distance to TSS be used to rank gRNAs for CRISPRa and CRISPRi applications? TRUE by default. For SpCas9 and human targets, this should be set to FALSE if addCrispraiScores was used.

## Details

For each nuclease, we rank gRNAs based on several rounds of priority. For SpCas9, gRNAs with unique target sequences and without 1- or 2-mismatch off-targets located in coding regions are placed into the first round. Then, gRNAs with a small number of one- or two-mismatch off-targets (less than 5) are placed into the second round. Remaining gRNAs are placed into the third round. Finally, any gRNAs overlapping a common SNP (human only), containing a polyT stretch, or with extreme GC content (below 20) are placed into the fourth round.

If `tx_id` is specified, within each round of selection, gRNAs targeting the first 85 percent of the specific transcript are prioritized first. If `tx_id` is specified, and `commonExon` is set to `TRUE`, gRNAs targeting common exons across isoforms are also prioritized. If a conservation score is available, gRNAs targeting conserved regions (phyloP conservation score greater than 0), are also prioritized.

Within each bin, gRNAs are ranked by a composite on-target activity rank to prioritize active gRNAs. The composite on-target activity rank is calculated by taking the average rank across the DeepHF and DeepSpCas9 scores for CRISPRko. For CRISPRa or CRISPRi, the CRISPRai scores are used if available.

The process is identical for enAsCas12a, with the exception that the enPAMGb method is used as the composite score.

For CasRx, gRNAs targeting all isoforms of a given gene, with no 1- or 2-mismatch off-targets, are placed into the first round. gRNAs targeting at least 50 percent of the isoforms of a given gene, with no 1- or 2-mismatch off-targets, are placed into the second round. Remaining gRNAs are placed into the third round. Within each round of selection, gRNAs are further ranked by the CasRxRF on-target score.

## Value

A `GuideSet` object ranked from best to worst gRNAs, with a column rank stored in `mcols(guideSet)` indicating gRNA rank.

## Author(s)

Luke Hoberecht, Jean-Philippe Fortin

## Examples

```
data(guideSetExampleFullAnnotation, package="crisprDesign")
gs <- rankSpacers(guideSetExampleFullAnnotation,
                 tx_id = "ENST00000538872")
gs
```

**Description**

These objects are imported from other packages. Follow the links below to see their documentation.

**GenomeInfoDb** [seqnames](#)

**S4Vectors** [mcols](#)

---

removeRepeats	<i>Remove <a href="#">GuideSet</a> gRNAs that overlap repeat elements</i>
---------------	---

---

**Description**

Remove [GuideSet](#) gRNAs that overlap repeat elements.

**Usage**

```
removeRepeats(object, ...)

## S4 method for signature 'GuideSet'
removeRepeats(object, gr.repeats = NULL, ignore.strand = TRUE)

## S4 method for signature 'PairedGuideSet'
removeRepeats(object, gr.repeats = NULL, ignore.strand = TRUE)

## S4 method for signature '`NULL`'
removeRepeats(object)
```

**Arguments**

object	A <a href="#">GuideSet</a> object or a <a href="#">PairedGuideSet</a> object.
...	Additional arguments, currently ignored.
gr.repeats	A <a href="#">GRanges</a> object containing repeat elements regions.
ignore.strand	Should gene strand be ignored when annotating? TRUE by default.

**Value**

object filtered for spacer sequences not overlapping any repeat elements. An inRepeats column is also appended in mcols(object).

**Author(s)**

Jean-Philippe Fortin, Luke Hoberecht

**See Also**

[link{addRepeats}](#).



## Examples

```
data(guideSetExample, package="crisprDesign")
data(grRepeatsExample, package="crisprDesign")
guideSet <- removeRepeats(guideSetExample,
                          gr.repeats=grRepeatsExample)
```

---

```
removeSpacersWithSecondaryTargets
      Remove gRNAs targeting secondary targets
```

---

## Description

Remove gRNAs targeting secondary targets

## Usage

```
removeSpacersWithSecondaryTargets(
  guideSet,
  geneID,
  geneColumn = "gene_id",
  ignoreGenesWithoutSymbols = TRUE,
  ignoreReadthroughs = TRUE
)
```

## Arguments

guideSet	A <a href="#">GuideSet</a> object.
geneID	String specifying gene ID of the main gene target.
geneColumn	Column in geneAnnotation(guideSet) specifying gene IDs
ignoreGenesWithoutSymbols	Should gene without gene symbols be ignored when removing co-targeting gRNAs?
ignoreReadthroughs	Should readthrough genes be ignored when removing co-targeting gRNAs?

## Details

The protospacer target sequence of gRNAs can be located in overlapping genes, and this function allows users to filter out such gRNAs. This ensures remaining gRNAs are targeting only one gene.

## Value

A [GuideSet](#) object with gRNAs targeting multiple targets removed.

## Author(s)

Jean-Philippe Fortin

---

tssObjectExample	<i>Example of a <a href="#">GRanges</a> object containing TSS coordinates</i>
------------------	---

---

**Description**

Example of a [GRanges](#) containing transcription starting site (TSS) coordinates for human gene IQSEC3 (ENSG00000120645).

**Usage**

```
data(tssObjectExample, package="crisprDesign")
```

**Format**

[GRanges](#) object of length 2 corresponding to the 2 TSSs of gene IQSEC3.

**Details**

The TSS coordinates were obtained from the two transcript stored in the `grListExample` object for gene IQSEC3.

---

TxDb2GRangesList	<i>Convert a <a href="#">TxDb</a> object into a <a href="#">GRangesList</a></i>
------------------	---

---

**Description**

Convenience function to reformat a [TxDb](#) object into a [GRangesList](#).

**Usage**

```
TxDb2GRangesList(
  txdb,
  standardChromOnly = TRUE,
  genome = NULL,
  seqlevelsStyle = c("UCSC", "NCBI")
)
```

**Arguments**

<code>txdb</code>	A <a href="#">TxDb</a> object.
<code>standardChromOnly</code>	Should only standard chromosomes be kept? TRUE by default.
<code>genome</code>	Optional string specifying genome. e.g. "hg38", to be added to <code>genome(txdb)</code> .
<code>seqlevelsStyle</code>	String specifying which style should be used for sequence names. "UCSC" by default (including "chr"). "NCBI" will omit "chr" in the sequence names.

**Value**

A named [GRangesList](#) of length 7 with the following elements: transcripts, exons, introns, cds, fiveUTRs, threeUTRs and tss.

**Author(s)**

Jean-Philippe Fortin, Luke Hoberecht

**See Also**

[getTxDb](#) to obtain a [TxDb](#) object.

**Examples**

```
if (interactive()){
  # To obtain a TxDb for Homo sapiens from Ensembl:
  txdb <- getTxDb()

  # To convert to a GRanges list:
  txdb <- TxDb2GRangesList(txdb)
}
```

---

updateOpsLibrary

*Update OPS library with additional gRNAs*

---

**Description**

Update OPS library with additional gRNAs

**Usage**

```
updateOpsLibrary(
  opsLibrary,
  df,
  n_guides = 4,
  gene_field = "gene",
  min_dist_edit = 2,
  dist_method = c("hamming", "levenshtein"),
  splitByChunks = FALSE
)
```

**Arguments**

opsLibrary	data.frame obtained from designOpsLibrary.
df	data.frame containing information about additional candidate gRNAs to add to the OPS library.
n_guides	Integer specifying how many gRNAs per gene should be selected. 4 by default.
gene_field	String specifying the column in df specifying gene names.
min_dist_edit	Integer specifying the minimum distance edit required for barcodes to be considered dissimilar. Barcodes that have edit distances less than the min_dist_edit will not be included in the library. 2 by default.
dist_method	String specifying distance method. Must be either "hamming" (default) or "levenshtein".
splitByChunks	Should distances be calculated in a chunk-wise manner? FALSE by default. Highly recommended when the set of query barcodes is large to reduce memory footprint.

**Value**

A data.frame containing the original gRNAs from the input opsLibrary data.frame as well as additional gRNAs selected from the input data.frame df.

**Author(s)**

Jean-Philippe Fortin

**Examples**

```
data(guideSetExample, package="crisprDesign")
guideSet <- unique(guideSetExample)
guideSet <- addOpsBarcodes(guideSet)
guideSet1 <- guideSet[1:200]
guideSet2 <- guideSet[201:400]

df1 <- data.frame(ID=names(guideSet1),
                  spacer=spacers(guideSet1, as.character=TRUE),
                  opsBarcode=as.character(guideSet1$opsBarcode))
df2 <- data.frame(ID=names(guideSet2),
                  spacer=spacers(guideSet2, as.character=TRUE),
                  opsBarcode=as.character(guideSet2$opsBarcode))

# Creating mock gene:
df1$gene <- rep(paste0("gene", 1:10), each=20)
df2$gene <- rep(paste0("gene", 1:10+10), each=20)
df1$rank <- rep(1:20, 10)
df2$rank <- rep(1:20, 10)
opsLib <- designOpsLibrary(df1)
opsLib <- updateOpsLibrary(opsLib, df2)
```

---

validateOpsLibrary	<i>Validate gRNA library for optical pooled screening</i>
--------------------	---

---

### Description

Validate gRNA library for optical pooled screening

### Usage

```
validateOpsLibrary(  
  df,  
  min_dist_edit = 2,  
  dist_method = c("hamming", "levenshtein")  
)
```

### Arguments

df	data.frame containing information about candidate gRNAs from which to build the OPS library. See details.
min_dist_edit	Integer specifying the minimum distance edit required for barcodes to be considered dissimilar.
dist_method	String specifying distance method. Must be either "hamming" (default) or "levenshtein".

### Value

The original df is all checks pass. Otherwise, a stop error.

### Author(s)

Jean-Philippe Fortin

### Examples

```
data(guideSetExample, package="crisprDesign")  
guideSet <- unique(guideSetExample)  
guideSet <- addOpsBarcodes(guideSet)  
df <- data.frame(ID=names(guideSet),  
                 spacer=spacers(guideSet, as.character=TRUE),  
                 opsBarcode=as.character(guideSet$opsBarcode))  
df$gene <- rep(paste0("gene", 1:40), each=20)  
df$rank <- rep(1:20, 40)  
opsLib <- designOpsLibrary(df)  
opsLib <- validateOpsLibrary(opsLib)
```

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