

# Package ‘multicrispr’

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**Title** Multi-locus multi-purpose Crispr/Cas design

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**Encoding** UTF-8

**Description** This package is for designing Crispr/Cas9 and Prime Editing experiments. It contains functions to (1) define and transform genomic targets, (2) find spacers (4) count offtarget (mis)matches, and (5) compute Doench2016/2014 targeting efficiency. Care has been taken for multicrispr to scale well towards large target sets, enabling the design of large Crispr/Cas9 libraries.

**License** GPL-2

**LazyData** true

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**Depends** R (>= 4.0)

**Imports** assertive, BiocGenerics, Biostrings, BSgenome, CRISPRseek, data.table, GenomeInfoDb, GenomicFeatures, GenomicRanges, ggplot2, grid, karyoploteR, magrittr, methods, parallel, plyranges, Rbowtie, reticulate, rtracklayer, stats, stringi, tidyr, tidyselect, utils

**Suggests** AnnotationHub, BiocStyle, BSgenome.Hsapiens.UCSC.hg38, BSgenome.Mmusculus.UCSC.mm10, BSgenome.Scerevisiae.UCSC.sacCer1, ensemblDb, IRanges, knitr, magick, rmarkdown, testthat, TxDb.Mmusculus.UCSC.mm10.knownGene

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**Author** Aditya Bhagwat [aut, cre],  
 Rene Wiegandt [ctb],  
 Mette Bentsen [ctb],  
 Jens Preussner [ctb],  
 Michael Lawrence [ctb],  
 Hervé Pagès [ctb],  
 Johannes Graumann [sad],  
 Mario Looso [sad, rth]

**Maintainer** Aditya Bhagwat <aditya.bhagwat@mpi-bn.mpg.de>

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add_genome_matches	<i>Add genome matches</i>
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---

## Description

Add genome matches

**Usage**

```
add_genome_matches(  
  spacers,  
  bsgenome = getBSgenome(genome(spacers)[1]),  
  mismatches = 2,  
  pam = "NGG",  
  offtargetmethod = c("bowtie", "pdict")[1],  
  outdir = OUTDIR,  
  indexedgenomesdir = INDEXEDGENOMESDIR,  
  verbose = TRUE  
)
```

**Arguments**

spacers	GRanges
bsgenome	BSgenome
mismatches	number
pam	string
offtargetmethod	'bowtie' or 'pdict'
outdir	bowtie output directory
indexedgenomesdir	directory with indexed genomes
verbose	TRUE (default) or FALSE

**Value**

GRanges

**Examples**

```
require(magrittr)  
file <- system.file('extdata/SRF.bed', package='multicrispr')  
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10  
targets0 <- bed_to_granges(file, 'mm10')  
targets <- extend(targets0)  
spacers <- find_spacers(targets, bsgenome, complement = FALSE,  
                        ontargetmethod = NULL, offtargetmethod = NULL)  
spacers %<>% extract(1:100)  
spacers %<>% add_genome_matches(bsgenome)
```

---

add\_inverse\_strand     *Add inverse strand*

---

## Description

Add inverse strand

## Usage

```
add_inverse_strand(gr, verbose = FALSE, plot = FALSE, ...)
```

## Arguments

gr	<a href="#">GRanges-class</a>
verbose	TRUE or FALSE (default)
plot	TRUE or FALSE (default)
...	<a href="#">plot_intervals</a> arguments

## Value

[GRanges-class](#)

## Examples

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                       HBB  = 'chr11:5227002:-',        # snp
                       HEXA  = 'chr15:72346580-72346583:-', # del
                       CFTR  = 'chr7:117559593-117559595:+', # ins
                       bsgenome)
add_inverse_strand(gr, plot = TRUE)

# TFBS example
#-----
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, genome = 'mm10')
add_inverse_strand(gr)
```

---

add_seq	<i>Add sequence to GRanges</i>
---------	--------------------------------

---

## Description

Add sequence to GRanges

## Usage

```
add_seq(gr, bsgenome, verbose = FALSE, as.character = TRUE)
```

## Arguments

gr	<a href="#">GRanges-class</a>
bsgenome	<a href="#">BSgenome-class</a>
verbose	TRUE or FALSE (default)
as.character	TRUE (default) or FALSE

## Value

[GRanges-class](#)

## Examples

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+', # snp
                       HBB = 'chr11:5227002:-', # snp
                       HEXA = 'chr15:72346580-72346583:-', # del
                       CFTR = 'chr7:117559593-117559595:+'), # ins
                     bsgenome)
(gr %<>% add_seq(bsgenome))

# TFBS example
#-----
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, 'mm10')
(gr %<>% add_seq(bsgenome))
```

---

add\_target\_matches     *Add target matches*

---

## Description

Add target matches

## Usage

```
add_target_matches(  
  spacers,  
  targets,  
  bsgenome,  
  mismatches = 2,  
  pam = "NGG",  
  outdir = OUTDIR,  
  verbose = TRUE  
)
```

## Arguments

spacers	GRanges
targets	GRanges
bsgenome	BSgenome
mismatches	number
pam	string
outdir	bowtie output directory
verbose	TRUE (default) or FALSE

## Value

GRanges

## Examples

```
require(magrittr)  
file <- system.file('extdata/SRF.bed', package='multicrispr')  
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10  
targets0 <- bed_to_granges(file, 'mm10')  
targets <- extend(targets0)  
spacers <- find_spacers(targets, bsgenome, complement = FALSE,  
  ontargetmethod = NULL, offtargetmethod = NULL)  
spacers %<>% add_target_matches(targets, bsgenome)
```

---

bed_to_granges	<i>Read bedfile into GRanges</i>
----------------	----------------------------------

---

## Description

Read bedfile into GRanges

## Usage

```
bed_to_granges(  
  bedfile,  
  genome,  
  txdb = NULL,  
  do_order = TRUE,  
  plot = TRUE,  
  verbose = TRUE  
)
```

## Arguments

bedfile	file path
genome	string: UCSC genome name (e.g. 'mm10')
txdb	NULL (default) or <a href="#">TxDb-class</a> (used for gene annotation)
do_order	TRUE (default) or FALSE: order on seqnames and star?
plot	TRUE (default) or FALSE: plot karyogram?
verbose	TRUE (default) or FALSE

## Value

[GRanges-class](#)

## See Also

[char\\_to\\_granges](#), [genes\\_to\\_granges](#)

## Examples

```
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')  
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10  
(gr <- bed_to_granges(bedfile, genome='mm10'))
```

---

char_to_granges	<i>Convert character vector into GRanges</i>
-----------------	--

---

**Description**

Convert character vector into GRanges

**Usage**

```
char_to_granges(x, bsgenome)
```

**Arguments**

x	character vector
bsgenome	<a href="#">BSgenome-class</a>

**Value**

[GRanges-class](#)

**See Also**

[bed\\_to\\_granges](#), [genes\\_to\\_granges](#)

**Examples**

```
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
x <- c(PRNP = 'chr20:4699600:+', # snp
      HBB = 'chr11:5227002:-', # snp
      HEXA = 'chr15:72346580-72346583:-', # del
      CFTR = 'chr7:117559593-117559595:+') # ins
gr <- char_to_granges(x, bsgenome)
plot_intervals(gr, facet_var = c('targetname', 'seqnames'))
```

---

double_flank	<i>Double flank</i>
--------------	---------------------

---

**Description**

Double flank



**Usage**

```
double_flank(
  gr,
  upstart = -200,
  upend = -1,
  downstart = 1,
  downend = 200,
  strandaware = TRUE,
  plot = FALSE,
  linetype_var = "set",
  ...
)
```

**Arguments**

gr	<a href="#">GRanges-class</a>
upstart	upstream flank start in relation to start(gr)
upend	upstream flank end in relation to start(gr)
downstart	downstream flank start in relation to end(gr)
downend	downstream flank end in relation to end(gr)
strandaware	TRUE (default) or FALSE
plot	TRUE or FALSE (default)
linetype_var	gr var mapped to linetype
...	passed to plot_intervals

**Value**

[GRanges-class](#)

**Examples**

```
# Prime Editing example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+', # snp
                       HBB = 'chr11:5227002:-', # snp
                       HEXA = 'chr15:72346580-72346583:-', # del
                       CFTR = 'chr7:117559593-117559595:+'), # ins
                     bsgenome)
double_flank(gr, -10, -1, +1, +20, plot = TRUE)

# TFBS example
#-----
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, genome = 'mm10', plot = FALSE)
double_flank(gr, plot = TRUE)
```

---

extend\_for\_pe                      *Extend ranges for prime editing*

---

## Description

Extend target ranges to span in which to look for spacer-pam seqs

## Usage

```
extend_for_pe(
  gr,
  bsgenome,
  nrt = 16,
  spacer = strrep("N", 20),
  pam = "NGG",
  plot = FALSE
)
```

## Arguments

gr	<a href="#">GRanges-class</a>
bsgenome	<a href="#">BSgenome-class</a>
nrt	number: reverse transcription length
spacer	string: spacer pattern in extended IUPAC alphabet
pam	string: pam pattern in extended IUPAC alphabet
plot	TRUE (default) or FALSE

## Details

Extend target ranges to find nearby spacers for prime editing

## Value

[GRanges-class](#)

## Examples

```
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c( PRNP = 'chr20:4699600:+',          # snp
                        HBB  = 'chr11:5227002:-',          # snp
                        HEXA = 'chr15:72346580-72346583:-', # del
                        CFTR = 'chr7:117559593-117559595:+'), # ins
                    bsgenome = bsgenome)
find_primespacers(gr, bsgenome)
(grext <- extend_for_pe(gr))
find_spacers(grext, bsgenome, complement = FALSE)
```

---

extend\_pe\_to\_gg      *Extend prime editing target to find GG sites*

---

## Description

Extend prime editing target to find GG sites in accessible neighbourhood

## Usage

```
extend_pe_to_gg(gr, nrt = 16, plot = FALSE)
```

## Arguments

gr	target <a href="#">GRanges-class</a>
nrt	n RT nucleotides (default 16, recommended 10-16)
plot	TRUE or FALSE (default)

## Details

Extends each target range to the area in which to search for a prime editing GG duplet, as shown in the sketch below.

```
=====>---GG--->---GG---> ** <---GG---<---GG---<=====
```

## Value

[GRanges-class](#)

## Examples

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+', # snp
                       HBB = 'chr11:5227002:-', # snp
                       HEXA = 'chr15:72346580-72346583:-', # del
                       CFTR = 'chr7:117559593-117559595:+'), # ins
                     bsgenome)
extend_pe_to_gg(gr, plot = TRUE)
```

---

extract\_matchranges    *Extract matching subranges*

---

## Description

Extract subranges that match pattern

## Usage

```
extract_matchranges(gr, bsgenome, pattern, plot = FALSE)
```

## Arguments

gr	<a href="#">GRanges-class</a>
bsgenome	<a href="#">BSgenome{BSgenome-class}</a>
pattern	string: search pattern in extended IUPAC alphabet
plot	TRUE or FALSE (default)

## Value

[GRanges-class](#)

## Examples

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                       HBB  = 'chr11:5227002:-',         # snp
                       HEXA  = 'chr15:72346580-72346583:-', # del
                       CFTR  = 'chr7:117559593-117559595:+', # ins
                       bsgenome))

gr %<>% extend_for_pe()
pattern <- strrep('N',20) %>% paste0('NGG')
extract_matchranges(gr, bsgenome, pattern, plot = TRUE)

# TFBS examples
#-----
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, 'mm10') %>% extend()
extract_matchranges(gr, bsgenome, pattern = strrep('N',20) %>% paste0('NGG'))
```

---

extract_subranges	<i>Extract subranges</i>
-------------------	--------------------------

---

**Description**

Extract subranges from a [GRanges-class](#) object

**Usage**

```
extract_subranges(gr, ir, plot = FALSE)
```

**Arguments**

gr	<a href="#">GRanges-class</a>
ir	<a href="#">IRanges-class</a> : subranges to be extracted
plot	TRUE or FALSE (default)

**Value**

[GRanges-class](#).

**Examples**

```
# Extract a subrange
gr <- GenomicRanges::GRanges(c(A = 'chr1:1-100:+', B = 'chr1:1-100:-'))
gr$targetname <- 'AB'
ir <- IRanges::IRanges(c(A = '1-10', A = '11-20', B = '1-10', B = '11-20'))
extract_subranges(gr, ir, plot = TRUE)

# Return empty GRanges for empty IRanges
extract_subranges(GenomicRanges::GRanges('chr1:345-456'), IRanges::IRanges())
```

---

find_gg	<i>Find GG</i>
---------	----------------

---

**Description**

Find GG

**Usage**

```
find_gg(gr)
```

**Arguments**

gr	<a href="#">GRanges-class</a>
----	-------------------------------

**Value**

GRanges-class

**Examples**

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+', # snp
                       HBB = 'chr11:5227002:-', # snp
                       HEXA = 'chr15:72346580-72346583:-', # del
                       CFTR = 'chr7:117559593-117559595:+'), # ins
                     bsgenome)
gr %<>% extend_pe_to_gg(plot = TRUE) %>% add_seq(bsgenome)
find_gg(gr)
```

---

find_primespacers	<i>Find prime editing spacers</i>
-------------------	-----------------------------------

---

**Description**

Find prime editing spacers around target ranges

**Usage**

```
find_primespacers(
  gr,
  bsgenome,
  edits = get_plus_seq(bsgenome, gr),
  nprimer = 13,
  nrt = 16,
  ontargetmethod = c("Doench2014", "Doench2016")[1],
  offtargetmethod = c("bowtie", "pdict")[1],
  mismatches = 0,
  nickmatches = 2,
  indexedgenomesdir = INDEXEDGENOMESDIR,
  outdir = OUTDIR,
  verbose = TRUE,
  plot = TRUE,
  ...
)
```

**Arguments**

gr	GRanges-class
bsgenome	BSgenome-class

edits	character vector: desired edits on '+' strand. If named, names should be identical to those of gr
nprimer	n primer nucleotides (default 13, max 17)
nrt	n rev transcr nucleotides (default 16, recomm. 10-16)
ontargetmethod	'Doench2014' or 'Doench2016': on-target scoring method
offtargetmethod	'bowtie' or 'pdict'
mismatches	no of primespacer mismatches (default 0, to suppress offtarget analysis: -1)
nickmatches	no of nickspacer offtarget mismatches (default 2, to suppresses offtarget analysis: -1)
indexedgenomesdir	directory with indexed genomes (as created by <a href="#">index_genome</a> )
outdir	directory where offtarget analysis output is written
verbose	TRUE (default) or FALSE
plot	TRUE (default) or FALSE
...	passed to plot_intervals

## Details

Below the architecture of a prime editing site. Edits can be performed anywhere in the revtranscript area.

```
spacer pam -----=== primer revtranscript -----===== 1.....17....GG.....
.....CC..... -----extension-----
```

## Value

[GRanges-class](#) with prime editing spacer ranges and following mcols: \* crisprspacer: N20 spacers \* crisprpam: NGG PAMs \* crisprprimer: primer (on PAM strand) \* crisprtranscript: reverse transcript (on PAM strand) \* crisprextension: 3' extension of gRNA contains: reverse transcription template + primer binding site sequence can be found on non-PAM strand \* crisprexrange: genomic range of crispr extension \* Doench2016|4: on-target efficiency scores \* off0, off1, off2: number of offtargets with 0, 1, 2 mismatches \* off: total number of offtargets: off = off0 + off1 + ... \* nickrange: nickspacer range \* nickspacer: nickspacer sequence \* nickDoench2016|4: nickspacer Doench scores \* nickoff: nickspacer offtarget counts

## See Also

[find\\_spacers](#) to find standard crispr sites

## Examples

```
# Find PE spacers for 4 clinically relevant loci (Anzalone et al, 2019)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(
  PRNP = 'chr20:4699600:+',          # snp: prion disease
  HBB  = 'chr11:5227002:-',        # snp: sickle cell anemia
```

```

    HEXA = 'chr15:72346580-72346583:-', # del: tay sachs disease
    CFTR = 'chr7:117559593-117559595:+'), # ins: cystic fibrosis
    bsgenome)
  spacers <- find_primespacers(gr, bsgenome)
  spacers <- find_spacers(extend_for_pe(gr), bsgenome, complement = FALSE)

# Edit PRNP locus for resistance against prion disease (Anzalone et al, 2019)
  bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
  gr <- char_to_granges(c(PRNP = 'chr20:4699600:+'), bsgenome)
  find_primespacers(gr, bsgenome)
  find_primespacers(gr, bsgenome, edits = 'T')

```

---

 find\_spacers

*Find crispr spacers in targetranges*


---

## Description

Find crispr spacers in targetranges

## Usage

```

find_spacers(
  gr,
  bsgenome,
  spacer = strrep("N", 20),
  pam = "NGG",
  complement = TRUE,
  ontargetmethod = c("Doench2014", "Doench2016")[1],
  offtargetmethod = c("bowtie", "pdict")[1],
  offtargetfilterby = character(0),
  subtract_targets = FALSE,
  mismatches = 2,
  indexedgenomesdir = INDEXEDGENOMESDIR,
  outdir = OUTDIR,
  verbose = TRUE,
  plot = TRUE,
  ...
)

```

## Arguments

gr	<a href="#">GRanges-class</a>
bsgenome	<a href="#">BSgenome-class</a>
spacer	string: spacer pattern in extended IUPAC alphabet
pam	string: pam pattern in extended IUPAC alphabet
complement	TRUE (default) or FALSE: also search in compl ranges?
ontargetmethod	'Doench2016', 'Doench2016' or NULL (no on-target score)



```

offtargetmethod
    'bowtie', 'pdict', or NULL (no offtarget analysis)
offtargetfilterby
    filter for best off-target counts by this variable
subtract_targets
    TRUE or FALSE (default): whether to subtract target (mis)matches from offtar-
    get counts
mismatches
    0-3: allowed mismatches in offtargetanalysis (choose mismatch=-1 to suppress
    offtarget analysis)
indexedgenomesdir
    directory with Bowtie-indexed genomes (as produced with index\_genome)
outdir
    directory where bowtie analysis results are written to
verbose
    TRUE (default) or FALSE
plot
    TRUE (default) or FALSE
...
    passed to plot_intervals

```

**Value**

[GRanges-class](#)

**See Also**

[find\\_primespacers](#) to find prime editing spacers

**Examples**

```

# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+', # snp
                       HBB = 'chr11:5227002:-', # snp
                       HEXA = 'chr15:72346580-72346583:-', # del
                       CFTR = 'chr7:117559593-117559595:+'), # ins
                     bsgenome)

plot_intervals(gr)
find_primespacers(gr, bsgenome)
find_spacers(extend_for_pe(gr), bsgenome, complement=FALSE, mismatches=0)
# complement = FALSE because extend_for_pe already
# adds reverse complements and does so in a strand-specific
# manner

# TFBS example
#-----
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, 'mm10') %>% extend()
gr %<>% extract(1:100)
find_spacers(gr, bsgenome, subtract_targets = TRUE)

```

---

genes\_to\_granges      *Convert geneids into GRanges*

---

## Description

Convert geneids into GRanges

## Usage

```
genes_to_granges(geneids, txdb, complement = TRUE, plot = TRUE, verbose = TRUE)
```

```
genefile_to_granges(file, txdb, complement = TRUE, plot = TRUE)
```

## Arguments

geneids	Gene identifier vector
txdb	<a href="#">TxDb-class</a> or <a href="#">EnsDb-class</a>
complement	TRUE (default) or FALSE: add complementary strand?
plot	TRUE (default) or FALSE
verbose	TRUE (default) or FALSE
file	Gene identifier file (one per row)

## Value

[GRanges-class](#)

## See Also

[char\\_to\\_granges](#), [bed\\_to\\_granges](#)

## Examples

```
# Entrez
#-----
genefile <- system.file('extdata/SRF.entrez', package='multicrispr')
geneids  <- as.character(read.table(genefile)[[1]])
txdb     <- getFromNamespace('TxDb.Mmusculus.UCSC.mm10.knownGene',
                             'TxDb.Mmusculus.UCSC.mm10.knownGene')
(gr <- genes_to_granges(geneids, txdb))
(gr <- genefile_to_granges(genefile, txdb))

# Ensembl
#-----
# txdb <- AnnotationHub::AnnotationHub()[["AH75036"]]
# genefile <- system.file('extdata/SRF.ensembl', package='multicrispr')
# geneids <- as.character(read.table(genefile)[[1]])
# (gr <- genes_to_granges(geneids, txdb))
# (gr <- genefile_to_granges(genefile, txdb))
```

---

gr2dt	<i>GRanges</i> <-> <i>data.table</i>
-------	--------------------------------------

---

**Description**

GRanges <-> data.table

**Usage**

```
gr2dt(gr)
```

```
dt2gr(dt, seqinfo)
```

**Arguments**

gr	<a href="#">GRanges-class</a>
dt	data.table
seqinfo	<a href="#">Seqinfo-class</a>

**Value**

data.table (gr2dt) or GRanges (dt2gr)

**Examples**

```
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+', # snp
                       HBB = 'chr11:5227002:-', # snp
                       HEXA = 'chr15:72346580-72346583:-', # del
                       CFTR = 'chr7:117559593-117559595:+'), # ins
                     bsgenome)

(dt <- gr2dt(gr))
(gr <- dt2gr(dt, BSgenome::seqinfo(bsgenome)))
```

---

has_been_indexed	<i>Has been indexed?</i>
------------------	--------------------------

---

**Description**

Has been indexed?

**Usage**

```
has_been_indexed(bsgenome, indexedgenomesdir = INDEXEDGENOMESDIR)
```

**Arguments**

bsgenome           BSgenome  
 indexedgenomesdir  
                     directory with indexed genomes

**Value**

TRUE or FALSE

**Examples**

```
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
has_been_indexed(bsgenome)
```

---

index_genome	<i>Index genome</i>
--------------	---------------------

---

**Description**

Bowtie index genome

**Usage**

```
index_genome(  
  bsgenome,  
  indexedgenomesdir = INDEXEDGENOMESDIR,  
  download = TRUE,  
  overwrite = FALSE  
)
```

**Arguments**

bsgenome           [BSgenome-class](#)  
 indexedgenomesdir  
                     string: directory with bowtie-indexed genome  
 download           TRUE (default) or FALSE: whether to download pre-indexed version if available  
 overwrite          TRUE or FALSE (default)

**Details**

Checks whether already available locally. If not, checks whether indexed version can be downloaded from our s3 storage. If not, builds the index with bowtie. This can take a few hours, but is a one-time operation.

**Value**

invisible(genomdir)

## Examples

```
bsgenome <- BSgenome.Scerevisiae.UCSC.sacCer1::Scerevisiae
index_genome(bsgenome, indexedgenomesdir = tempdir())
```

---

index_targets	<i>Index targets</i>
---------------	----------------------

---

## Description

Bowtie index targets

## Usage

```
index_targets(
  targets,
  bsgenome = getBSgenome(genome(targets)[1]),
  outdir = OUTDIR,
  verbose = TRUE
)
```

## Arguments

targets	<a href="#">GRanges-class</a>
bsgenome	<a href="#">BSgenome-class</a>
outdir	string: output directory
verbose	TRUE (default) or FALSE

## Value

invisible(targetdir)

## Examples

```
require(magrittr)
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')
targets <- extend.bed_to_granges(bedfile, genome = 'mm10')
index_targets(targets, bsgenome)
```

---

plot\_intervals      *Interval plot GRanges*

---

### Description

Interval plot GRanges

### Usage

```
plot_intervals(
  gr,
  xref = "targetname",
  y = default_y(gr),
  nperchrom = 2,
  nchrom = 4,
  color_var = "targetname",
  facet_var = "seqnames",
  linetype_var = default_linetype(gr),
  size_var = default_size_var(gr),
  alpha_var = default_alpha_var(gr),
  title = NULL,
  scales = "free"
)
```

### Arguments

gr	<a href="#">GRanges-class</a>
xref	gr var used for scaling x axis
y	'names' (default) or name of gr variable
nperchrom	number (default 1): n head (and n tail) targets shown per chromosome
nchrom	number (default 6) of chromosomes shown
color_var	'seqnames' (default) or other gr variable
facet_var	NULL(default) or gr variable mapped to facet
linetype_var	NULL (default) or gr variable mapped to linetype
size_var	NULL (default) or gr variable mapped to size
alpha_var	NULL or gr variable mapped to alpha
title	NULL or string: plot title
scales	'free', 'fixed', etc

### Value

ggplot object

**See Also**[plot\\_karyogram](#)**Examples**

```

# SRF sites
require(magrittr)
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')
targets <- bed_to_granges(bedfile, 'mm10', plot = FALSE)
plot_intervals(targets)

# PE targets
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',
                       HBB = 'chr11:5227002:-',
                       HEXA = 'chr15:72346580-72346583:-',
                       CFTR = 'chr7:117559593-117559595:+'),
                     bsgenome)
spacers <- find_primespacers(gr, bsgenome, plot = FALSE)
plot_intervals(gr)
plot_intervals(extend_for_pe(gr))
plot_intervals(spacers)

# Empty gr
plot_intervals(GenomicRanges::GRanges())

```

plot\_karyogram

*Karyo/Interval Plot GRanges(List)***Description**

Karyo/Interval Plot GRanges(List)

**Usage**

```
plot_karyogram(grlist, title = unique(genome(grlist)))
```

**Arguments**

grlist	<a href="#">GRanges-class</a>
title	plot title

**Value**

list

**See Also**[plot\\_intervals](#)**Examples**

```
# Plot GRanges
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')
gr <- bed_to_granges(bedfile, 'mm10', plot = FALSE)
plot_karyogram(gr)

# Plot GRangesList
flanks <- up_flank(gr, stranded=FALSE)
grlist <- GenomicRanges::GRangesList(sites = gr, flanks = flanks)
plot_karyogram(grlist)
```

---

score_ontargets	<i>Add on-target efficiency scores</i>
-----------------	--

---

**Description**

Add Doench2014 or Doench2016 on-target efficiency scores

**Usage**

```
score_ontargets(
  spacers,
  bsgenome,
  ontargetmethod = c("Doench2014", "Doench2016")[1],
  chunksize = 10000,
  verbose = TRUE,
  plot = TRUE,
  ...
)
```

**Arguments**

spacers	<a href="#">GRanges-class</a> : spacers
bsgenome	<a href="#">BSgenome-class</a>
ontargetmethod	'Doench2014' (default) or 'Doench2016' (requires non-NULL argument python, virtualenv, or condaenv)
chunksize	Doench2016 is executed in chunks of chunksize
verbose	TRUE (default) or FALSE
plot	TRUE (default) or FALSE
...	passed to <a href="#">plot_intervals</a>



## Details

add\_ontargets adds efficiency scores filter\_ontargets adds efficiency scores and filters on them

## Value

numeric vector

## References

Doench 2014, Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. Nature Biotechnology, doi: 10.1038/nbt.3026

Doench 2016, Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nature Biotechnology, doi: 10.1038/nbt.3437

Python module azimuth: [github/MicrosoftResearch/azimuth](https://github.com/MicrosoftResearch/azimuth)

## Examples

```
# Install azimuth
#-----
## With reticulate
# require(reticulate)
# conda_create('azienv', c('python=2.7'))
# use_condaenv('azienv')
# py_install(c('azimuth', 'scikit-learn==0.17.1', 'biopython==1.76'),
#           'azienv', pip = TRUE)

## Directly
# conda create --name azienv python=2.7
# conda activate azienv
# pip install scikit-learn==0.17.1
# pip install biopython==1.76
# pip install azimuth

# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
targets <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                           HBB = 'chr11:5227002:-',          # snp
                           HEXA = 'chr15:72346580-72346583:-', # del
                           CFTR = 'chr7:117559593-117559595:+'), # ins
                          bsgenome)

spacers <- find_primespacers(targets, bsgenome, ontargetmethod=NULL,
                             offtargetmethod=NULL)
spacers %<>% score_ontargets(bsgenome, 'Doench2014')
# reticulate::use_condaenv('azienv')
# reticulate::import('azimuth')
# spacers %<>% score_ontargets(bsgenome, 'Doench2016')

# TFBS example
```

```
#-----
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
targets <- extend(bed_to_granges(bedfile, 'mm10'))
spacers <- find_spacers(targets, bsgenome, ontargetmethod=NULL,
                        offtargetmethod=NULL)
spacers %<>% score_ontargets(bsgenome, 'Doench2014')
# reticulate::use_condaenv('azienv')
# reticulate::import('azimuth')
# spacers %>% score_ontargets(bsgenome, 'Doench2016')
```

---

up\_flank

*Extend or Flank GRanges*


---

### Description

Returns extensions, upstream flanks, or downstream flanks

### Usage

```
up_flank(
  gr,
  start = -200,
  end = -1,
  strandaware = TRUE,
  bsgenome = NULL,
  verbose = FALSE,
  plot = FALSE,
  linetype_var = "set",
  ...
)
```

```
down_flank(
  gr,
  start = 1,
  end = 200,
  strandaware = TRUE,
  bsgenome = NULL,
  verbose = FALSE,
  plot = FALSE,
  linetype_var = "set",
  ...
)
```

```
extend(
  gr,
  start = -22,
  end = 22,
```

```

    strandaware = TRUE,
    bsgenome = NULL,
    verbose = FALSE,
    plot = FALSE,
    linetype_var = "set",
    ...
)

```

## Arguments

gr	<a href="#">GRanges-class</a>
start	number or vector (same length as gr): start definition, relative to gr start (up_flank, extend) or gr end (down_flank).
end	number or vector (same length as gr): end definition, relative to gr start (up_flank) or gr end (extend, down_flank).
strandaware	TRUE (default) or FALSE: consider strand information?
bsgenome	NULL (default) or <a href="#">BSgenome-class</a> . Required to update gr\$seq if present.
verbose	TRUE or FALSE (default)
plot	TRUE or FALSE (default)
linetype_var	string: gr var mapped to linetype
...	passed to <a href="#">plot_intervals</a>

## Details

up\_flank returns upstream flanks, in relation to start(gr). down\_flank returns downstream flanks, in relation to end(gr). extend returns extensions, in relation to start(gr) and end(gr)

## Value

a [GRanges-class](#)

## Examples

```

# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                       HBB = 'chr11:5227002:-',          # snp
                       HEXA = 'chr15:72346580-72346583:-', # del
                       CFTR = 'chr7:117559593-117559595:+'), # ins
                     bsgenome = bsgenome)
gr %>% up_flank( -22, -1, plot=TRUE)
gr %>% up_flank( c(-10,-20,-30,-40), -1, plot=TRUE)
gr %>% up_flank( -22, -1, plot=TRUE, strandaware=FALSE)

gr %>% down_flank(+1, +22, plot=TRUE)
gr %>% down_flank(+1, c(10, 20, 30, 40), plot=TRUE)

```

```

gr %>% down_flank(+1, +22, plot=TRUE, strandaware=FALSE)

gr %>% extend( -10, +20, plot=TRUE)
gr %>% extend( -10, +20, plot=TRUE, strandaware=FALSE)

# TFBS example
#-----
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, genome = 'mm10')
gr %>% extend(plot = TRUE)
gr %>% up_flank(plot = TRUE)
gr %>% down_flank(plot = TRUE)

```

---

write\_ranges

*Write GRanges to file*


---

## Description

Write GRanges to file

## Usage

```
write_ranges(gr, file, verbose = TRUE)
```

```
read_ranges(file, bsgenome)
```

## Arguments

gr	<a href="#">GRanges-class</a>
file	file
verbose	TRUE (default) or FALSE
bsgenome	<a href="#">BSgenome-class</a>

## Value

[GRanges-class](#) for read\_ranges

## Examples

```

# Find PE spacers for 4 clinically relevant loci (Anzalone et al, 2019)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(
  PRNP = 'chr20:4699600:+',          # snp: prion disease
  HBB = 'chr11:5227002:-',          # snp: sickle cell anemia
  HEXA = 'chr15:72346580-72346583:-', # del: tay sachs disease
  CFTR = 'chr7:117559593-117559595:+'), # ins: cystic fibrosis
  bsgenome)
file <- file.path(tempdir(), 'gr.txt')
write_ranges(gr, file)
read_ranges(file, bsgenome)

```

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